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Effects of Plant Population on Soil Structure, Soil Moisture Depletion and on Yield of Cassava (*Manihot esculenta*) on an Ultisol in Southeast Nigeria

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ABSTRACT

The effects of four cassava populations (8, 10, 13 and 20×10³ plants per ha⁻¹) on soil physical properties, moisture retention characteristics, moisture potential profiles and moisture depletion patterns were compared with uncropped plots receiving no cultivation of an Ultisol in the humid region in eastern Nigeria. The growing of cassava decreased the bulk density of 0–10 cm depth by 17% in comparison with the uncultivated soil (1·53 Mg m⁻³). The bulk density of the 10–20 cm depth was higher for the cropped than the uncropped control plots. The moisture retention below a suction of pF 2·4 for 0–10 cm depth was less for the uncultivated control than for soil growing cassava. The reverse was found in moisture retention characteristics of the 20–40 cm depth especially for low suction ranges of pF below 0·8. The 0–10 cm soil layer of uncropped control dried more rapidly than the cropped soil. Below 10 cm, however, cassava depleted moisture reserves more rapidly than in uncropped control. The effect of plant population on soil moisture depletion was more conspicuous within than between rows. Significant differences in water flux and in water uptake from different horizons were observed among various population densities. The total water loss was the highest for the least plant population for the between-row zone, and for the highest population for the within-row zone. Plant population had no effect on tuber and stalk yield.

Key words: Plant population, soil structure, soil moisture, cassava, Ultisol.

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1 INTRODUCTION

Optimum plant population of cassava depends on edaphic and climatic factors, variety, soil fertility, cultural practices, and the purpose of utilisation of the roots.[1–3] The length of the growing season, determined mainly by soil moisture conditions, decides the time to plant and the associated crops for the mixed-crop cultivation for cassava production in subsistence farming.[4] Cassava has a prolonged growing season, the production of one crop usually extending beyond the end of the period of high soil moisture. Soil-moisture regime from the end of the annual moisture cycle to the time when the tubers are harvested may, therefore, affect the production potential of cassava in some agro-ecological regions. Recent studies[5] indicated that the longer the period of high soil moisture, in proportion to the growing season from planting to harvest, the higher the yield. The production potential declines in increasingly dry areas.[6] Significant yield reductions occur under conditions of frequent drought and when the crop is grown on soils of low available water-holding capacity.[7,8] The choice of optimum plant population is, therefore, an important agronomic measure in cassava production for effective utilisation of the root zone space and of the available soil-moisture.

The available research information with respect to optimum plant population and yields of cassava is difficult to generalise because it is soil and climate specific. In general, cassava yield on low-fertility soils depends on the varietal characteristics.[9] A population range of 10 000–20 000 plants ha^{-1} is found to be optimum, depending on soil fertility and management systems.[10–17] There is, however, a need to identify the optimum plant population for major cassava cultivars for both monoculture and intercropping systems for different soils and agro-ecological regions of Africa.

The interaction between soil and the processes of tuberous root development, though not well understood, is known to cause some improvement in physical properties of about 0–15 cm surface soil.[18] The longer the ground cover provided by canopy the more favourable the effect. The effectiveness of the cover also depends on variety and plant population. Lower plant densities encourage weed growth, reduce production per unit area, and have less beneficial effects on the soil.

The objectives of this investigation, therefore, were to evaluate the effects of plant population of cassava on soil structure, on moisture depletion patterns at the end of the annual rainfall cycle, and on the yield of cassava for an Ultisol in southeastern Nigeria.

2 EXPERIMENTAL

2.1 Site description

This experiment was conducted at Onne, an IITA substation located in the high-rainfall region of southern Nigeria, during the 1982–83 season. The climate of this region is humid and supports a lowland tropical rainforest vegetation. The mean annual rainfall is about 2500 mm, generally falling between the beginning of

March and November. The experimental site was cleared of secondary forest during the early part of 1980. The soil at Onne is an Ultisol (derived from coastal sediments), and is classified as loamy siliceous, isohyperthermic, oxic paleudult. Some chemical and physical properties of the soil are shown in Table 1. The soil is acidic and has a pH ranging between 4·5 at 0–10 cm depth to about 5·4 at 60–80 cm depth. Soil organic carbon content ranges from 1·17 to 0·19%, total nitrogen from 0·11 to 0·04%, total acidity from 2·05 to 1·69 meq 100 g^{-1} and effective cation-exchange capacity (ECEC) from 2·59 to 1·92 meq 100 g^{-1} between 0–10 and 80–100 cm depths, respectively. The sand content decreases with depth with a corresponding increase in clay content.

TABLE 1
Some Chemical and Physical Properties of the Experimental Soil

Depth (cm)	*pH*	*Organic carbon (%)*	*Total nitrogen (%)*	*Total acidity (meq 100 g^{-1})*	*ECEC[a] (meq 100 g^{-1})*	*Particle size distribution (%)*			*Saturated hydraulic conductivity (cm day^{-1})*
						Sand	*Silt*	*Clay*	
0–10	4·5	1·17	0·113	2·05	2·57	67·6	6·0	26·6	2494
10–20	4·8	0·50	0·059	1·79	2·20	63·6	4·0	32·4	3103
20–40	4·8	0·33	0·072	1·98	2·31	59·6	4·0	36·4	1550
40–60	4·9	0·28	0·056	1·61	1·97	57·6	4·0	48·4	1742
60–80	5·4	0·33	0·053	1·72	2·11	57·6	4·0	38·4	1317
80–100	5·0	0·19	0·044	1·69	1·92	55·6	4·0	40·4	1388

[a]ECEC, effective cation-exchange capacity.

2.2. Field layout

The experimental layout was a complete randomised block with three replications. Each replicate (30×8 m) consisted of four cultivated plots with a spacing of 0·5×1·0, 0·75×1·0, 1·0×1·0 and 1·25×1.0 m within and between rows to give a population of 20 000, 13 300, 10 000 and 8000 plants ha^{-1}, respectively. In addition, a fifth bare uncultivated plot (4×4 m) was maintained in each block. The bare plots were last ploughed in March 1981. Immediately after harvesting the 1981–82 crops, the plots were mowed, ploughed to a depth of approximately 20 cm and harrowed. Planting was done on flats and the cuttings were inserted into the ground at an angle of about 40° and to a depth of about 8–10 cm. Planting was done on the 2 April 1982. Weed control was achieved through one or two manual weedings when required. A buffer of 5 m was maintained between the cassava and control plot to minimise the shading and avoid rainfall interference by the cassava canopy.

2.3 Analytical methods

Soil bulk density was measured on undisturbed cores (5 cm long and 5 cm internal diameter). Three core samples were taken from each replicate of cultivated cropped and uncultivated bare plot from 0–10, 10–20 and 20–40 cm depths. These samples, obtained three weeks before harvesting in March 1983, were also used for measuring moisture retention characteristics using tension table and pressure plate extractors. Penetrometer resistance measurements were made for the sur-

face soil using a pocket penetrometer. Ten measurements were taken from each replicate, in March 1983, when the soil was dry.

Tensiometric measurements of soil water-suction were made at 10, 30, 60 and 90 cm depths using suction gauge tensiometers. One tensiometer was installed at each depth within and between the rows in each replicate. For bare plot, three tensiometers were installed at each depth and about 20 cm apart. A daily reading of suction was obtained throughout the growing season. Soil moisture samples for gravimetric moisture content determinations were taken weekly within and between the rows. Samples were taken from one location, each within and in between the rows of each replicate, and from three locations in each of the uncultivated bare plots from 0–15, 15–45, 45–60 and 60–90 cm depths. Gravimetric moisture content was converted into volumetric moisture content using the corresponding bulk density of each depth. Soil moisture content data were plotted as a function of time during a continuous drying phase (after the cessation of rain on 8 December 1982).

The crop was harvested on 12 April 1983, 12 months after planting. Each plot was harvested completely and all tubers were counted, and tubers and stalks were weighed. Tuber lengths and circumferences were measured on 10 representative samples from each replicate.

3. RESULTS AND DISCUSSION

3.1 Bulk density and penetrometer resistance

The effects of plant population on soil bulk density is shown in Table 2. For 0–10 cm depth, plant population had no significant effect on soil bulk density. In 10–20 cm depth, there was also no significant difference in soil bulk density

TABLE 2
The Effects of Plant Population on Soil Bulk Density of Three Soil Layers (0–10, 10–20 and 20–40 cm Depths) and Penetrometer Resistance of 0–10 cm Depth

Treatment		*Bulk density ($Mg\ m^{-3}$)*			*Penetrometer resistance[a] (MPa)*
Spacing (m)	*Plant population ha^{-1}*	*0–10*	*10–20*	*20–40*	
0·5×1	20 000	1·32a	1·54a	1·42a	0·293a[b]
0·75×1	13 300	1·28a	1·49ab	1·43a	0·290a
1×1	10 000	1·33a	1·47ab	1·43a	0·290a
1·25×1	8 000	1·30a	1·51a	1·48a	0·285a
Uncultivated bare		1·53b	1·42b	1·45a	—
Mean		1·35	1·49	1·45	0·290
LSD (0·05)		0·15	0·07	0·10	0·028
CV (%)		5·84	2·65	3·77	11·61

[a]Resistance was measured when the soil was dry.
[b]Means within the same column followed by the same letter are not significantly different at the 5% level of probability using the new Duncan's Multiple Range Test.

between cultivated treatments, but higher bulk densities were measured for cultivated than uncultivated soil. The bulk densities of 20–40 cm depth were not affected by treatments imposed. Significant difference in soil bulk densities were observed between uncultivated bare and cultivated plots for 0–10 cm depth. The highest bulk density of 1·53 Mg m^{-3} was 17% more than the mean soil bulk density of the cultivated soil. A high bulk density of the 0–10 cm layer of the uncultivated bare plot may be due to compaction and crusting of the surface layer by rain-drop impact, which is less in cassava plots due to the protective effect of its canopy. Secondly, loosening of the surface layer of the soil by development of cassava tubers may have resulted in lower bulk densities, when compared with bare plot. Soil beneath the tubers may be compacted by the growth of the tubers, resulting in high bulk density. There were no differences in the penetrometer resistance of the surface layer treatments.

3.2 Moisture retention characteristics

The data in Fig. 1 show the moisture retention curves for the 0–10, 10–20 and 20–40 cm depths for the four cassava population treatments and the uncultivated bare plots. Significant differences in moisture retention occurred for some suctions among cultivated treatments and between cultivated and uncultivated bare plots. Moisture retention in 0–10 cm depth was the least for uncultivated bare plots for the suction range of pF<2·4, but moisture content values at saturation were not significantly different between treatments. The higher moisture retention for soils under cassava may be due to improvement in volume of macropores as a result of soil loosening by tuber development and to subsoil being brought to the surface by ploughing. For the higher suction range (pF>3·2), the moisture retention capacities of cultivated plots, though significantly different from those of bare uncultivated plots, did not differ among various population treatments. Moisture retention characteristics of the 0–20 cm layers were similar to those of the 0–10 cm depth. The effect of plant population on moisture retention capacities for the higher suction range (pF>3·2) was, however, more pronounced. In this suction range, soils from the bare plot had significantly lower moisture retention than soils with plant population of 20 000 and 8000 plants ha^{-1}. For 20–40 cm, the moisture retention characteristics of soil from the bare plot were significantly higher than those cultivated for low suction range (pF<0·8). For a suction range of pF<2·0, moisture retention capacity of uncultivated bare soil, though not significantly different, was lower than those of soils under cassava.

3.3 Soil moisture extraction

The effects of plant population on soil moisture suction for 10 and 30 cm depths, after cessation of rains are shown in Fig. 2. For 10 cm depth, suction was higher in bare plot during the first 5 to 7 days after cessation of rain than within and between-row suctions under cassava. Subsequently, higher suctions were recorded in soils under cassava than in bare plots. For example, suctions of 65 and 80 kPa were recorded 12 days after cessation of rain for uncultivated and cultivated plots, respectively. The effects of plant population on soil moisture suction

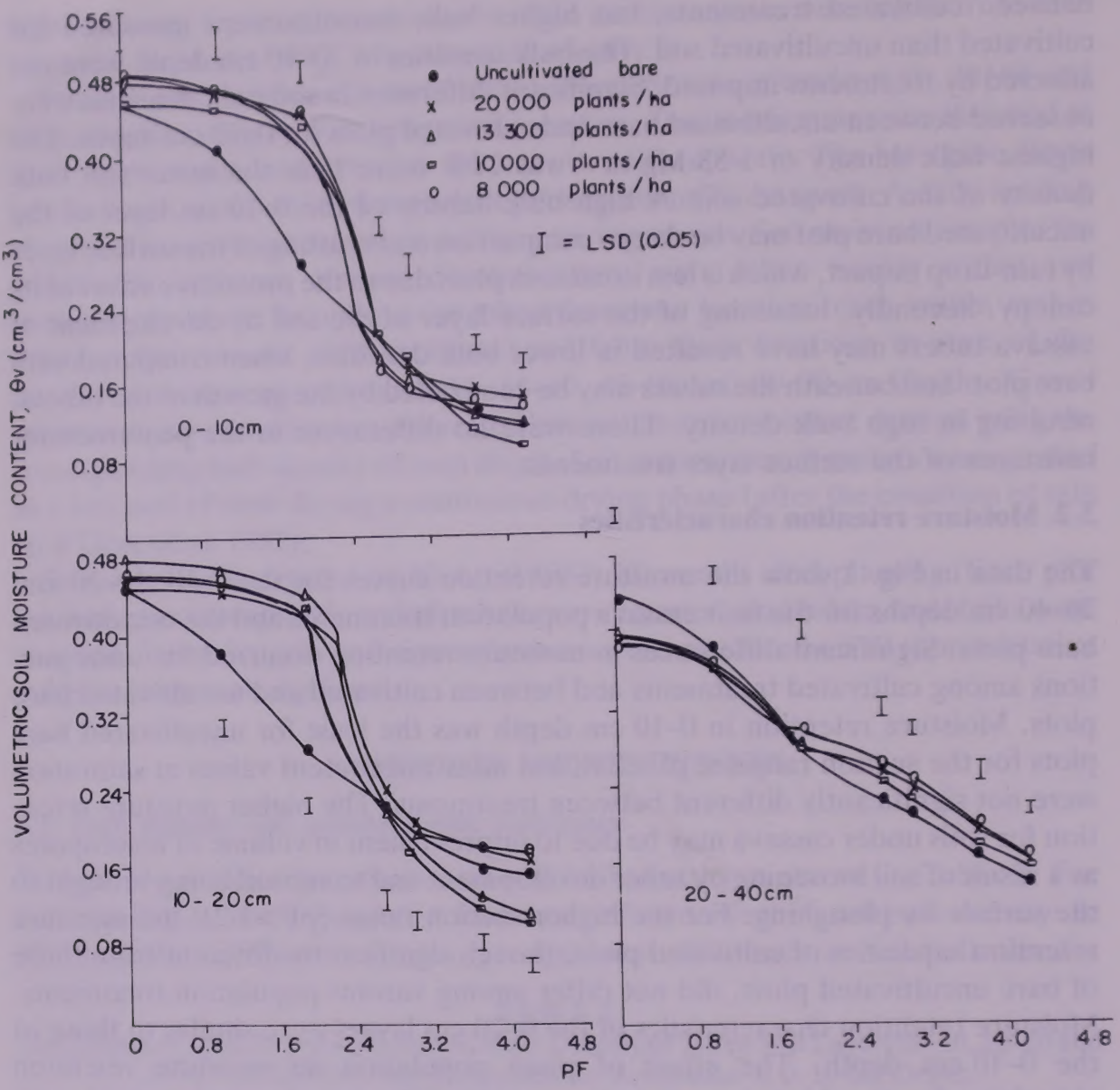

Fig. 1. Moisture retention curves for 0–10, 10–20 and 20–40 cm depths for soil sampled from plots of different population density.

was more conspicuous within than between rows. After 14 December, suctions increased more rapidly at high populations of 20 000 and 13 300 plants ha^{-1} than low populations of 10 000 and 8000 plants ha^{-1}. Soil water suctions were generally uniform for all cassava populations and for both between- and within-rows measurements for 30 cm depth. The soil moisture suctions recorded 21 days after rain were 80 and 38 kPa for cultivated and uncultivated plots, respectively.

The data in Fig. 3 relating the effects of plant population on volumetric moisture content in 0–15 and 15–45 cm soil layers on different days after rain show no variation in moisture content for between- and within-row locations. In 0–15 cm depth, there was no significant difference in moisture content due to plant population for both between- and within-row space. However, moisture reserves depleted slowly in the between-row zone for 20 000 plants ha^{-1} than for 13 300 plants ha^{-1}. Moisture contents for plots with 10 000 and 8000 plants ha^{-1} were in between those with 20 000 and 13 000 plants ha^{-1}. In the within-row

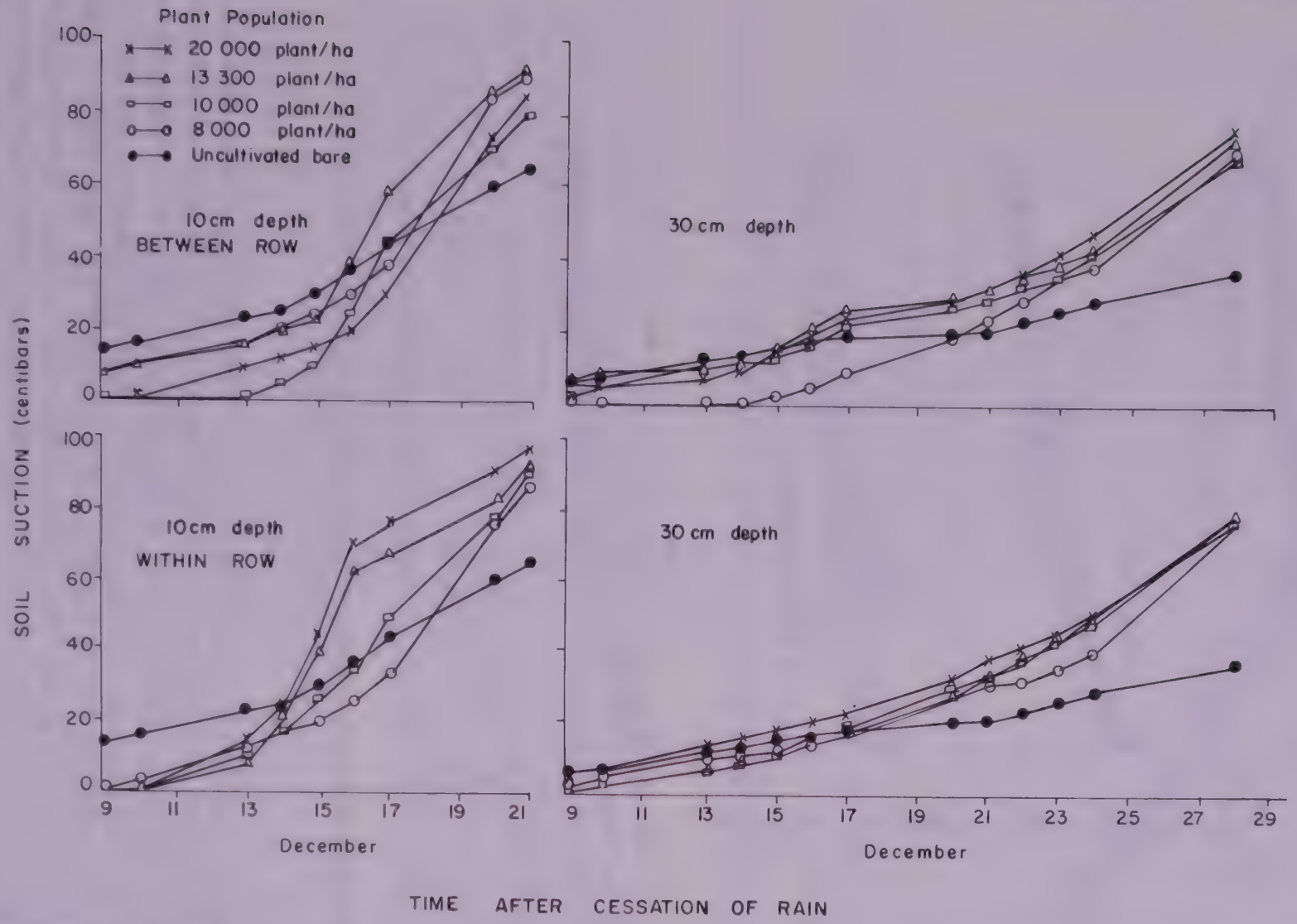

Fig. 2. The effect of cassava population on soil moisture suction at 10 and 30 cm depth, during December 1982 after cessation of rains.

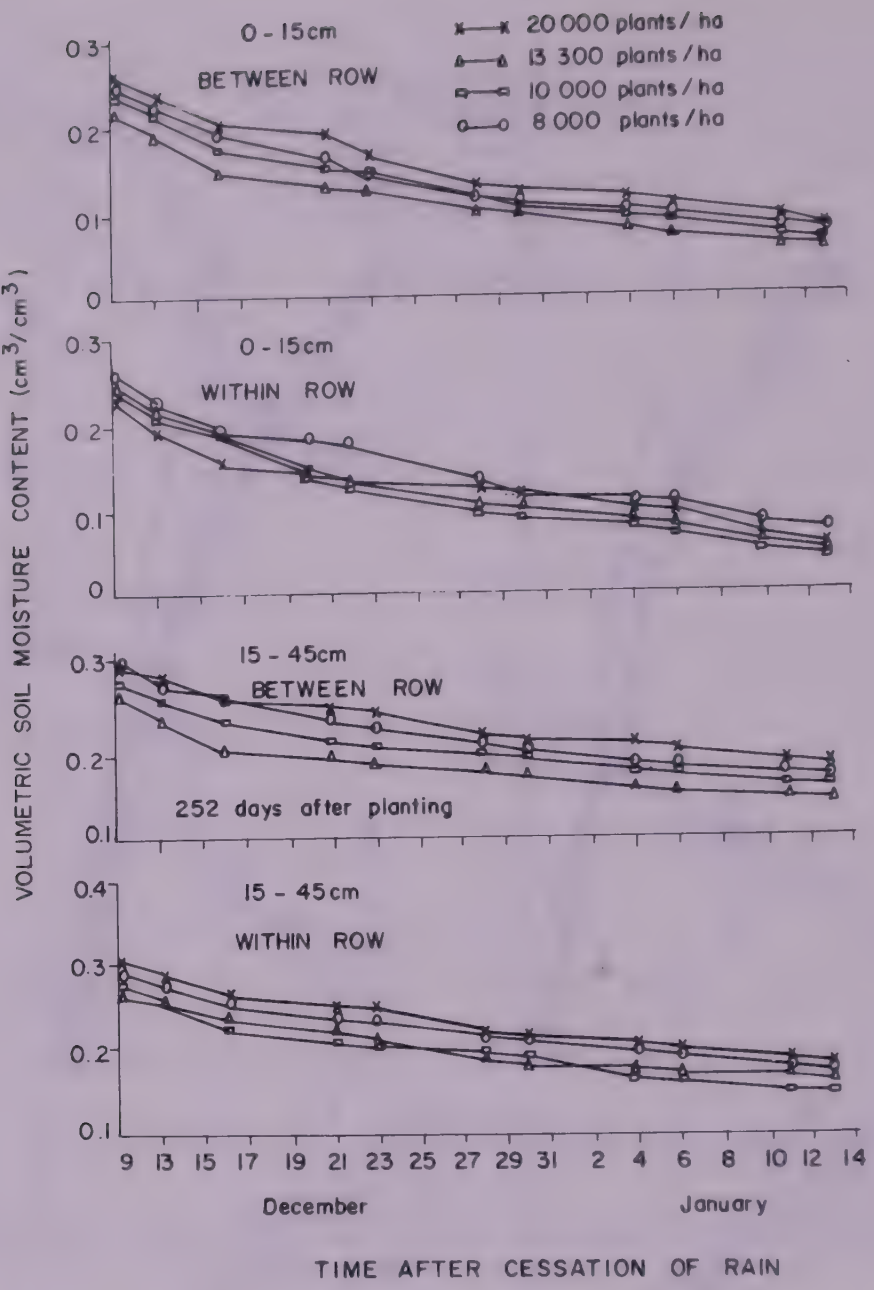

Fig. 3. Effects of cassava population on within- and between-row soil moisture content for 0–15 cm depth, and 15–45 cm depth.

position, however, plots with 8000 plants ha^{-1} had the highest moisture reserve. Significant differences in moisture content due to plant population were observed in 15–45 cm depth. Moisture content depleted slowly under 20 000 plants ha^{-1} in both the between- and within-row zones. A complete ground cover under the highest plant population minimised the evaporative loss even with increased severity of the dry season. The least moisture reserve under 13 000 plants ha^{-1} population appears to be related to better development of tuberous roots and to high evaporative loss, presumably due to a more open canopy which allowed evaporation to occur within as well as on the surface of the canopy.

Soil water fluxes at 30 cm soil depth are shown in Fig. 4 for both between- and within-row zones. These fluxes were calculated from the plot of the water content

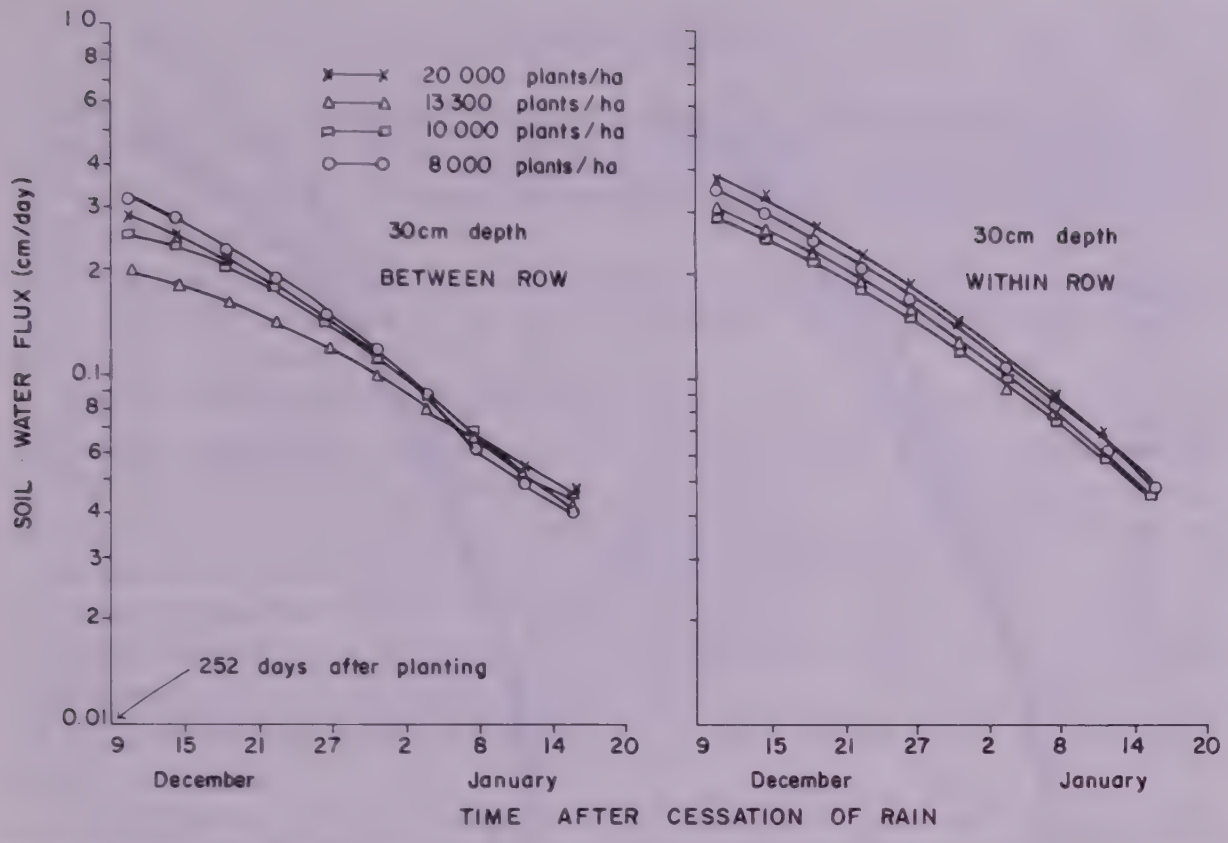

Fig. 4. Soil-water fluxes for different cassava populations during December 1982 and January 1983.

change with time. It is evident from the data in Fig. 4 that significant differences in fluxes due to plant population were evident during the initial periods of drying after the cessation of rain. Differences in fluxes were more for between- than within-row zones. In the between-row zone, soil water flux was the highest for the 8000 plants ha^{-1} and the least for the 13 300 plants ha^{-1}. For the within-row zone, however, the flux was the highest for the 20 000 plants ha^{-1} and the least for the 10 000 plants ha^{-1}. These differences in flux are probably due to differential evapotranspiration among varying plant populations. The rate of evapotranspiration affects soil water fluxes within the root zone.[19,20]

The effects of plant population on total water depletion from 0 to 95 cm depth at different times for between- and within-row zones is shown in Fig. 5. The data suggest that total water loss was significantly affected by differences in plant population. The total water depletion even for the same population was different for between- and within-row zones. For the between-row zone, total water loss was the highest for the 8000 plants ha^{-1} and least for the 13 300 plants ha^{-1}. There were no significant differences in total water depletion for the population densities of 20 000 and 10 000 plants ha^{-1}. For the within-row zone, total water depletion was significantly higher for the 20 000 plants ha^{-1} than for other plant populations. The total water depletion for within-row zone was not significantly different among the 13 300, 10 000 and 8000 plants ha^{-1} treatments. The patterns of soil water fluxes in the 30 cm soil depth (Fig. 4) and the total soil water loss from the 0 to 95-cm soil profile suggest a close relationship between soil water flux and total water depletion.

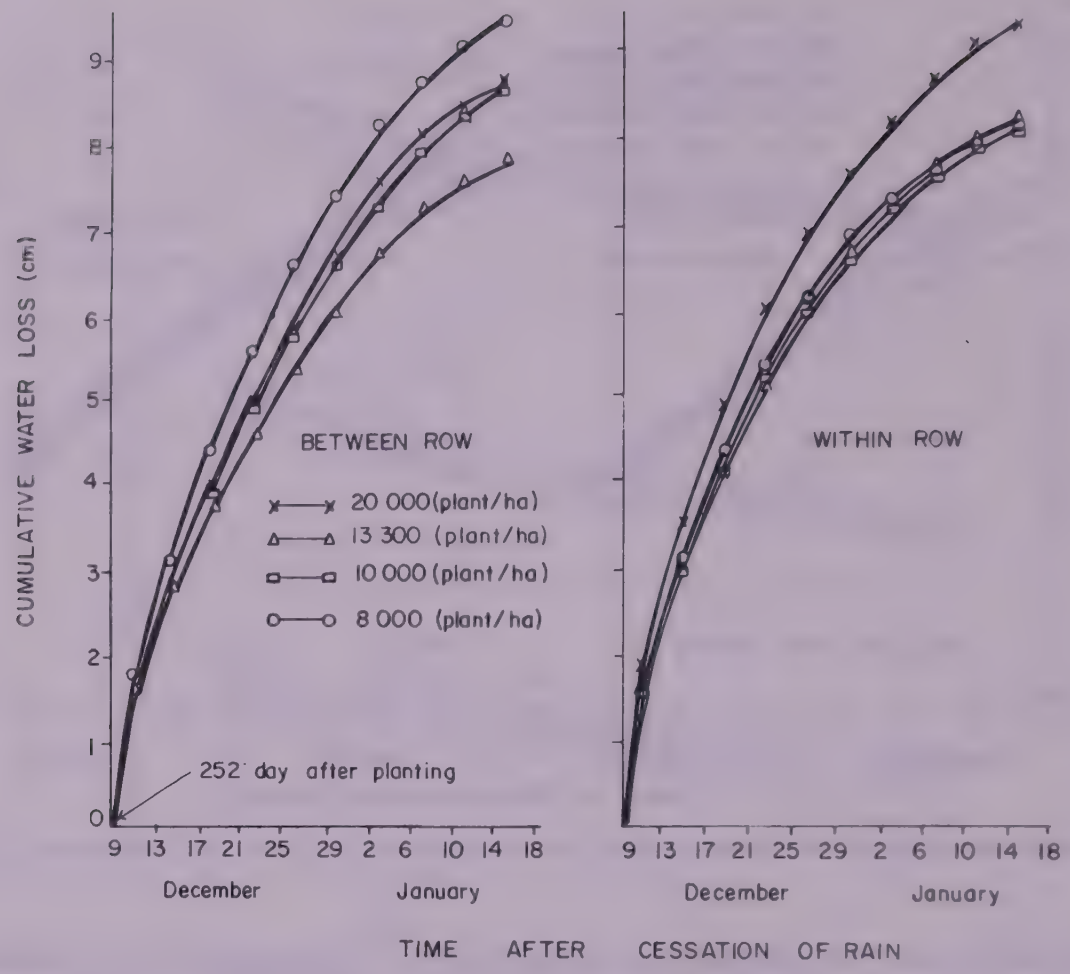

Fig. 5. Effects of cassava population on cumulative water depletion from 0 to 95 cm depth during December 1982 and January 1983.

3.4 Yield

The effects of plant population on the yield components of cassava harvested at 12-month growth are presented in Table 3. The data show that neither number of tubers, tuber yield, stalk yield nor average weight per tuber were affected by plant population.

TABLE 3
Effect of Plant Population on Fresh Tuber Yield of Cassava (1982/83)

Plant population (plant ha^{-1})	*Tubers ha^{-1}*	*Tuber yield (t ha^{-1})*	*Stalk yield (t ha^{-1})*	*Average weight/tuber (g)*
20 000	114 167	10·3	3·1	94·1
13 300	141 667	14·2	5·8	100·6
10 000	104 167	11·4	3·8	112·1
8 000	135 000	13·0	5·2	95·7
Mean	123 750	12·2	4·5	100·6
LSD (0·05)	56 704	5·2	3·1	48·9
CV (%)	22·9	21·4	34·3	24·3

4 CONCLUSIONS

Water depletion was higher under cassava than in uncultivated bare soil. While total porosity of the 0–20 cm depth did not change, the proportion of macropores (>14·3 μm) retaining water for the suction range pF<2·4 increased significantly by cassava cultivation.

There was a decrease in total porosity and the proportion of macropores (pF<1·0) under cassava in the 20–40 cm depth. The development of cassava tubers led to compaction of the soil beneath.

Soil water depletion was higher under cassava than for bare soil. The effects of high plant population on soil moisture depletion were more pronounced for within- than between-row zones. Soil water content was depleted to the lowest value under 20 000 plants ha^{-1} for both between- and within-row zones for the top 0–45 cm depth. Water fluxes and total water depletion were significantly affected by plant population. Significant differences in fluxes were observed during the initial periods of water depletion only, and fluxes were higher for between- than within-row zones. Low plant population (8000 plants ha^{-1}) depleted water faster in the between-row zones, while high population (20 000 plants ha^{-1}) did so in within-row zones.

Tuber yields and yield components did not differ significantly among plant populations studied.

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Seasonal Variations of Chemical Composition in Prickly Pear (*Opuntia ficus-indica* (L.) Miller)

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ABSTRACT

The moisture, ash, free reducing sugar, starch, ether extractive, crude protein, amino acid and the heat energy contents of young and mature cladodes of Opuntia ficus-indica, *with or without fruits, are described. The measurements were made in each of the following stages of development: vegetative growth (March), full vegetative development, and at the beginning of fructification (October). Independently of developmental stage (different cladode types) the highest values were reached always in young cladodes in the following months: moisture content in March and October; ash and free reducing sugars in March; starch and ether extractive in September and October; crude protein in March and July. Crude fibre was maximal in September and global heat energy in October. The nutritive and caloric value of cladodes are compared to other products used for livestock nutrition.*

Key words: Amino acids, cacti, CAM, cladodes, crude fibre, crude protein, fruits, heat energy, lipids, minerals, prickly pear, starch, sugar.

1 INTRODUCTION

Prickly pear (*Opuntia ficus-indica* L.) is a cactus in which the branches (cladodes) have the appearance and function of a leaf.[1] This plant assimilates CO_2 by Crassulacean Acid Metabolism (CAM) and is thus adapted to extreme arid conditions.[2] It can survive in areas with only 200 mm of annual rainfall[3] and grows normally with 300 mm of rain a year.[4] Physiological responses and CAM of *O. ficus-indica* have been studied using cultivated cacti.[4,5]

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Even though cladodes of cacti have been used on different occasions for animal and human nutrition,[6] their chemical composition is not well established. In some publications,[7,8,9] analytical results have been presented but not the methodology used, making comparison difficult. Recently, the amino and organic acids content of prickly pear has been reported.[10]

The nutritional value of prickly pear fruits for human consumption has been known for more than 2000 years.[11] The plant was brought to Europe by the first Spanish colonists from Mexico and has been cultivated along the Mediterranean coast since the late 17th Century.[11,12] Other uses described include medical, industrial, ornamental and as a source of fuel. During the last ten years, with the world energy crisis and the search for alternative solutions, there has been a revived interest in this plant and especially in its photosynthetic reception of solar energy in arid zones.[13]

Given the potential interest that the uses of the species *O. ficus-indica* present in the field of agroenergetics[13] and livestock nutrition,[7] the research described here focuses on the chemical composition of the different types of cladode that appear during a complete life cycle.

2 EXPERIMENTAL

2.1 Plant material

Different types of prickly pear *O. ficus-indica* cladodes (Y_1, young; Y_2, young with fruit; A_1 mature and A_2, mature with fruit), growing in Pelahustán (Toledo, Spain), were sampled through a complete life cycle in the manner shown in Table 1. The cladodes (dried at 60°C≥48 h) were ground in a Culatti hammer mill

TABLE 1
O. ficus-indica Cladodes: Developmental State and Sampling Dates During the Productive Cycle of Plants Grown in Toledo, Spain

Harvesting date	*Development*	*Plant material*[a]
March	Vegetative	Y_1, A_1, A_2
July	Flowering	Y_1, Y_2, A_1
September	Fruit-bearing	Y_1, A_1, A_2
October	Harvesting	Y_1, A_1, A_2

[a] Y_1, Young cladodes; Y_2, young cladodes with fruits; A_1, mature cladodes and A_2, mature cladodes with fruits.

(Mod. DFH 48) and the meal (particles smaller than 0·5 mm) was air dried at room temperature in hermetically closed glass containers (Mod. "Vac-Vem").

2.2 Water and mineral content

The moisture content of fresh cladodes and cladode meal was determined by drying at 130°C to constant weight. The cladodes were dried in two ways: in 20 mm diameter disks at 60°C and, in meal form, at 130°C.

The mineral content was determined by incineration of the meal at 600°C until the ash attained a greyish-white colour. The cation analysis (Na^+, K^+, Ca^{2+} and Mg^{2+}) was performed on the solution obtained by dissolving the samples of ash in nitric acid (HNO_3, 50% v/v) diluted with double distilled water (1:10). The results were determined on a Perkin–Elmer (Mod. 503) atomic absorption spectrophotometer using the characteristic emission (Na^+, K^+) and absorption (Ca^{2+}, Mg^{2+}) lines for this purpose.

2.3 Sugar analysis

The free reducing sugars were extracted from approximately 0·5 g meal with 80% v/v ethanol (50 ml) for 30 min twice. An aliquot of the alcoholic solution (0·1 ml) diluted with distilled water (1:10), was used for the determination of reducing sugars according to the methods of Nelson[14] and Somogyi.[15] A glucose solution (Merck) of 300 mg litre^{-1} was used as reference and the results (G_1) were expressed as glucose equivalents.

The starch content was determined after its hydrolysis to glucose with 1 M HCl at 95°C for 30 min.[16] Under these conditions the yield of glucose was >95%. The reducing sugar released was measured and converted into starch using the equation: $S=0{\cdot}9\,(G_2-G_1)/0{\cdot}95$ where G_1 and G_2 are reducing equivalents expressed as glucose before and after hydrolysis.

2.4 Ether extractives

Desiccated meal (5–10 g) was dried at 100°C and oil extracted with diethyl ether (boiling point 35°C) using a Soxhlet-type extractor for 4 h with a velocity of 4–5 drops s^{-1}.

2.5 Nitrogen and amino acid determination

Defatted cladode meal was digested following a modified semi-micro Kjeldahl method.[16] The ammonia was distilled into boric acid and titrated directly with standardised 0·1 N H_2SO_4 using methyl red solution as indicator. Protein was calculated as N×6·25.

The amino acid analyses were performed using a Technicon (TSM-1) amino acid analyser. Triplicate samples of defatted cladode meal (30 mg) were hydrolysed *in vacuo* with 6 M HCl (2 ml) for 24 h at 110°C. Tryptophan is completely destroyed under these conditions and a loss of sulphur-containing amino acids also occurs, resulting in very low analytical values, especially for cysteine.

2.6 Crude fibre determination

Crude fibre was considered to be the residue that remained after treating the defatted cladode meal with acid (H_2SO_4, 1·25% v/v) and alkaline (KOH, 5% v/v) solutions, for 30 min at 95–100°C, with intermediate washings with distilled water. The residue obtained was washed with ethanol (96% v/v) followed by diethyl ether, dried and weighed and then incinerated at 550°C. Crude fibre was determined as the difference in masses between the dry residue and the ash.

2.7 Heat energy determination

Heat energy was determined according to the method of Maynard and Loosli[17] using a Parr oxygen calorimeter charged with 0·5–1·0 g of sample and 25–30 atmospheres of oxygen. The sample was electrically ignited and temperature readings were taken on the thermometer to ascertain the maximum rise. This value multiplied by the sum of thermal capacity of the metal parts and the water mass gives the number of calories produced by the burning of the sample.

2.8 Calculations and statistical analysis

All of the values given are means from a minimum of three replicates. The data were submitted to an analysis of variance according to the following model:

$$X_{ijk}=\mu+A_i+B_{ij}+E_{ijk}$$

were μ is the general mean; A_i, is the effect of the ith season (March, July, September, October); B_{ij} is the effect of the jth cladode type within season (young and mature cladodes, with or without fruits) and E_{ijk} is the experimental error for the kth sample.

3 RESULTS AND DISCUSSION

The analysis of variance, summarised in Table 2, for the chemical composition of the different components studied shows that there were significant differences throughout the year ($P \leq 0{\cdot}01$) for all components as well as for cladodes at different stages of development in each season.

Tables 3–10 show the chemical composition of different cladode types harvested during four sampling dates, along one complete vegetative cycle. With the exception of moisture content, all the results were obtained from dry cladode meal.

TABLE 2
Summarised Analysis of Variance for Different Components of Prickly Pear (*O. ficus-indica*) Cladodes During its Life Cycle and Different Developmental Stages

Analysis	*EMS*[a]	*F*[b]	
		Season	*Age of cladode*
Moisture	$3{\cdot}33\times10^{-2}$	272·0***	33·8***
Ash	187·40	10·8**	7·0**
Free reducing sugars	1·65	1995·7***	1221·6***
Starch	19·25	603·4***	284·1***
Ether extractive	23·09	16·8**	5·7**
Crude protein	8·17	265·0***	492·9***
Crude fibre	33·99	184·4***	136·7***
Heat energy	2064·65	4·4*	5·5**

[a]Error mean square for treatment means.
[b]*F* values with upper level of significance: $P \leq 0{\cdot}05$ (*), $P \leq 0{\cdot}01$ (**) and $P \leq 0{\cdot}001$ (***).

TABLE 3
Water and Mineral Content (Mean±SE) in Cladodes of Prickly Pear (*O. ficus-indica*)

Harvesting date	*Origin of plant material*[a]				*Mean*
	Y_1	Y_2	A_1	A_2	
(A) Moisture (mg g^{-1} f.w.):					
March	952±3	—	937±1	923±2	937±4
July	931±6	905±1	904±3	—	914±5
September	875±3	—	828±4	886±2	863±9
October	924±4	—	920±5	929±1	924±2
(B) Ash (mg g^{-1} d.w.):					
March	298±7	—	294±4	251±11	281±9
July	244±14	254±3	247±7	—	248±5
September	220±4	—	252±1	273±15	248±9
October	264±4	—	246±2	262±7	257±3

[a]See Table 1.

TABLE 4
Cation Composition (mg g^{-1} ash) in Cladodes of Prickly Pear (*O. ficus-indica*) Harvested in October

Cation and relationship	*Origin of plant material*[a]		
	Y_1	A_1	A_2
Sodium (Na)	4	4	3
Potassium (K)	176	172	209
Calcium (Ca)	202	179	165
Magnesium (Mg)	93	96	84
Na+K+Ca+Mg	475	451	461
Ca/Mg	2·2	1·9	2·0
K/Na	44·0	43·0	69·7

[a]See Table 1.

TABLE 5
Analysis (Mean±SE) of Sugar Content (mg g^{-1} d.w.) in Cladodes of Prickly Pear (*O. ficus-indica*)

Harvesting date	*Origin of plant material*[a]				*Mean*
	Y_1	Y_2	A_1	A_2	
(A) Free sugars with reducing groups:					
March	40±1	—	103±2	93±1	79±10
July	38±1	79±1	64±1	—	60±6
September	10±1	—	18±1	70±1	33±9
October	64±1	—	67±1	59±1	63±1
(B) Starch:					
March	83±2	—	77±1	94±1	85±3
July	158±1	138±3	110±2	—	135±7
September	226±1	—	99±1	105±4	143±21
October	199±2	—	188±5	126±2	171±11

[a]See Table 1.

TABLE 6
Analysis (Mean±SE) of Ether Extractive Content (mg g^{-1} d.w.) in Cladodes of Prickly Pear (*O. ficus-indica*)

Harvesting date	*Origin of plant material*[a]				*Mean*
	Y_1	Y_2	A_1	A_2	
March	13±2	—	14±2	7±1	11±1
July	24±2	20±1	17±2	—	20±1
September	19±2	—	31±1	26±3	25±2
October	36±3	—	15±4	24±6	25±4

[a]See Table 1.

TABLE 7
Analysis (Mean±SE) of Crude Protein Content (mg g^{-1} d.w.) in Prickly Pear (*O. ficus-indica*)

Harvesting date	*Origin of plant material*[a]				*Mean*
	Y_1	Y_2	A_1	A_2	
March	148±1	—	92±1	72±1	104±11
July	167±2	76±1	75±1	—	106±15
September	105±1	—	47±4	79±1	77±9
October	126±1	—	111±1	100±1	112±4

[a]See Table 1.

TABLE 8
Amino Acid Composition in Cladodes of Prickly Pear (*O. ficus-indica*) Harvested in October

Amino acid	*Composition (mg g^{-1} protein as N×6·25)*
Aspartic acid+asparagine	111·9
Threonine	65·6
Serine	53·5
Glutamic acid+glutamine	171·3
Proline	66·8
Glycine	77·8
Alanine	49·8
Valine	81·4
Methionine	9·7(approx.)
Isoleucine	42·5
Leucine	80·2
Tyrosine	46·2
Phenylalanine	65·6
Lysine	35·2
Histidine	<1·0
Arginine	42·5
Total	1000·0

TABLE 9
Analysis (Mean±SE) of Crude Fibre (mg g $^{-1}$ d.w.) in Cladodes of Prickly Pear (*O. ficus-indica*)

Harvesting date	*Origin of plant material*[a]				*Mean*
	Y_1	Y_2	A_1	A_2	
March	119±2	—	122±1	154±1	132±6
July	107±1	125±2	188±7	—	140±12
September	123±6	—	246±2	168±6	179±18
October	109±1	—	110±2	131±1	117±4

[a]See Table 1.

TABLE 10
Analysis (Mean±SE) of Heat Energy (kJ kg^{-1} d.w.) in Cladodes of Prickly Pear (*O. ficus-indica*)

Harvesting date	*Origin of plant material*[a]				*Mean*
	Y_1	Y_2	A_1	A_2	
March	13950±258	—	14579±88	14404±13	14308±121
July	14188±29	14492±67	14563±79	—	14413±67
September	14263±50	—	14417±29	14417±50	14367±33
October	14246±108	—	14713±21	14888±200	14617±117

[a]See Table 1.

The freshly harvested cladodes with the lowest moisture content (Table 3) were those picked in the summer months (July–September); especially the young cladodes (Y_1) (87·5%) and mature cladodes without fruit (A_1) (82·8%) harvested in September. However, the moisture content present in these cladodes was still considerably higher than those of different fibrous wastes (cereal straws, sugar cane, seed husks, dried animal wastes, and wood wastes) whose chemical composition and digestibility for ruminants have already been reported.[18]

With the exception of the cladodes harvested during the month of March, the chemical compositions of the samples averaged over cladode types given in Table 3 did not show significant differences ($P \leq 0{\cdot}01$). The levels found are similar to or slightly higher than those of various dried animal wastes,[19,20] and much higher than those for some cereal straws.[21,22] Of the total mineral content, sodium, potassium, calcium and magnesium cations (Table 4) made up almost 50% of the ash.

The highest values for free reducing sugars (Table 5) were found in the adult cladodes of the first sampling (March), while those cladodes without fruit that were harvested in September had the lowest values. The seasonal variations seen in the starch contents showed the opposite trend: the lowest values of starch coincided with the first samplings harvested in March and the highest values with the September and October samplings of young cladodes without fruit.

The ether extractive content (Table 6) constituted a very small fraction of all those analysed. With the exception of the cladodes harvested in March, the

samples did not show any significant differences ($P \leq 0{\cdot}01$) in the content of ether extractives.

Since the appropriate conversion factor for nitrogen to protein has not been determined for prickly pear, the factor 6·25 was used as recommended by FAO[23] (Table 7). In all the samplings made, the crude protein content of young cladodes without fruit was always greater than that of mature cladodes, with or without fruit. The crude protein values found were considerably higher than those present in other fibrous wastes of vegetable origin[18] (cereal straws, sugar cane, seed husks, etc.) but lower than in dried animal wastes.[18]

The amino acid profile (excluding tryptophan) of defatted cladode meal is given in Table 8. Because of destruction during hydrolysis, the analytical values for cysteine have also been omitted. Glutamic acid+glutamine and aspartic acid+asparagine were the most abundant amino acids and methionine and histidine the least. The low values for methionine probably result from losses during hydrolysis *in vacuo*. Nutritionally essential amino acids account for about 38% of the total protein.

The highest crude fibre values (Table 9) were obtained during the summer samplings (July and September) from adult cladodes without fruit. In all the samplings, the lowest contents always corresponded to the young cladodes without fruit. The mean values found in each season's samplings are comparable to those that have already been described[18] for some dried animal wastes but are lower than for cereal straws.

The heat energy values (Table 10) for the different types of cladode (13·950–14·888 kJ kg^{-1}) are very similar throughout, showing no significant differences ($P \leq 0{\cdot}05$), except for those harvested in October that have a higher mean caloric content.

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Agriculture Group Symposium
Nitrogen in Crops and Food

The following are summaries of the papers presented to a joint meeting of the Agriculture Group of the Society of Chemical Industry and the Fertilizer Society, held on 21 October 1986 at the Society of Chemical Industry, 14–15 Belgrave Square, London SW1X 8PS. The papers published here are entirely the responsibility of the authors and do not reflect the views of the Editorial Board of the Journal of the Science of Food and Agriculture.

Nitrate Levels in Field Vegetables

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Most of the nitrogen taken up by vegetables is assimilated as nitrate. Within the plant nitrate is reduced to ammonium which is incorporated into amino acids and then into more complex nitrogen compounds. Any large accumulation of nitrate in the plant can be taken as an excess of supply over the demand for further metabolism. The metabolism of nitrogen requires energy so nitrate may be expected to accumulate in periods of low light intensity. Any conditions which reduce the growth rate of the plant whilst maintaining the nitrogen supply will tend to increase nitrate levels in the plant whilst a reduced nitrate supply and a rapidly growing plant will lower the nitrate levels.

ADAS has recently carried out several nitrogen trials on crisp lettuce. The opportunity was taken to analyse the plants at harvest for nitrate content. The results from four trials were examined in detail. In only one of these trials did the nitrate level exceed 4000 mg kg^{-1} fresh weight which is the maximum permissible in some European countries. The 4000 mg kg^{-1} nitrate level was only found in crops which received 100 kg ha^{-1} nitrogen fertiliser above that required for optimum yield when all the nitrogen was applied at the time of planting. Splitting the nitrogen and applying some at two weeks after planting increased the nitrate levels in the plants.

In all the trials to a greater or lesser extent levels increased with increasing applications of nitrogen fertiliser. In the trials where the total nitrogen of the plant

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was measured, there was a good correlation between nitrate content and nitrogen content.

A trial on spinach with different rates and times of application of nitrogen produced similar results to those for lettuce. High rates of nitrogen fertiliser application increased nitrate levels, and splitting the application also increased the levels. However, no treatment gave levels exceeding 4000 mg kg^{-1} nitrate.

Other trials have examined the effects of different nitrogen sources on the nitrate content of lettuce and radish. Plants given ammonium sulphate had the lowest nitrate contents and those given sodium nitrate the highest content with the plants receiving ammonium nitrate being intermediate. The effect of type of fertiliser was small compared to the effect of the amount of nitrogen applied.

Other data were collected from variety trials. There were wide variations in nitrate levels in cabbage but less variation between lettuce varieties. All were below 4000 mg kg^{-1}.

The general picture is that levels of nitrate in field vegetables are low; only if excessively high rates of nitrogen fertiliser are applied, particularly if applied as late top-dressings, do levels approach 4000 mg kg^{-1}.

Investigations into the Uptake of Nitrate by Protected Lettuce and Celery

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There is concern about high levels of nitrate in food and water because of a possible link with medical health problems including methaemoglobinaemia in young babies and a form of cancer in adults. At present about two-thirds of total nitrate is ingested from food and most of this comes from vegetables. Lettuce has a greater ability to accumulate nitrate in its foliage than many other widely consumed vegetables and this crop, especially glasshouse varieties, has come in for particular scrutiny. Since some European countries have established maximum permissible nitrate levels in vegetables work was started in Autumn 1984 to gain information on nitrate levels in glasshouse lettuce, and to a lesser extent glasshouse celery grown in the UK, and to investigate the effect of crop management on leaf nitrate levels at harvest.

There were two main lines of investigation. The first concentrated on application of varying rates of five fertilisers containing different proportions of nitrogen in either ammonium or nitrate form. The second involved incorporation of the nitrification inhibitors nitrapyrin and dicyandiamide into the soil. A small amount of work was done on supplying nitrogen to a crop grown by nutrient film technique as either ammonium-nitrogen or nitrate-nitrogen and on ceasing nitro-

gen addition to the circulating solution up to three weeks before harvest. In addition information was obtained on variation in leaf nitrate levels between varieties and between different plant parts.

Where soil nitrate level was initially low addition of only a small amount of nitrogen fertiliser markedly increased leaf nitrate level. Increasing addition of a given fertiliser led to a relatively small further increase in leaf nitrate. At all sites the leaf level was increased by similar amounts irrespective of the nitrate content of the fertiliser used although there were indications that use of ammonium sulphate gave the lowest levels.

Both nitrapyrin and dicyandiamide incorporated separately from fertiliser and dicyandiamide when added as a blend with fertiliser granules substantially reduced nitrate levels in lettuce and celery. Reducing the solution nitrogen level of an NFT crop before harvest and substituting ammonium for nitrate reduced leaf nitrate level but marketable yield was unacceptably reduced.

Small but important differences occurred between varieties. Iceberg varieties contained particularly low levels. Nitrate levels in the older, outer leaves of both crops were much higher than in the younger leaves.

Leaf nitrate levels were substantially higher in lettuce crops harvested in December/January than at other times of the year. However, recommending that planting is delayed to allow harvesting after January is not a practical solution. Slightly harder leaf trimming would reduce nitrate levels if this could be done without serious weight loss.

The use of nitrification inhibitors successfully reduced leaf nitrate level but further work is needed to confirm that their use does not reduce crop yield. In the longer term the breeding of varieties with lower nitrate levels may provide the most acceptable solution.

Nitrate Content in Glasshouse Lettuce

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More than 300 lettuce samples were collected from commercial glasshouses at the time of harvest, together with soil samples (0–25 cm). Soil nitrate was determined according to the 1:2 volume extract method.

The nitrate content of glasshouse lettuce strongly depends on global radiation (Table 1). In summer the content is relatively low. The differences in daily total radiation between the end of May and the beginning of August are small, and so are the differences in nitrate content in this period. In winter the amount of

TABLE 1

The Influence, Per Period of Two Months, of the Global Radiation (x, J cm^{-2} 10 days^{-1}) Measured During 10 days Prior to Harvest on the Nitrate Content in Lettuce (y, mg NO_3 kg^{-1} fresh wt.)

Period	*Number of samples*	*Regression equation*	*Corr. coeff.*	$\bar{x}$	$\bar{y}$
June–July	39	$y=0x+1\,887$	0	20 453	1 909
Aug.–Sept.	44	$y=-0{\cdot}08x+3\,511$	−0·33	13 045	2 495
Oct.–Nov.	57	$y=-0{\cdot}25x+4\,451$	−0·44	4 163	3 394
Dec.–Jan.	60	$y=-0{\cdot}04x+3\,760$	−0·02	2 358	3 671
Feb.–March	61	$y=-0{\cdot}10x+3\,959$	−0·35	6 307	3 339
Apr.–May	50	$y=-0{\cdot}07x+3\,446$	−0·43	14 695	2 408
Total	311	$y=-0{\cdot}10x+3\,855$	−0·75	9 928	2 965

radiant energy received on the northern hemisphere is low, and the nitrate content of crops is relatively high. Large differences in radiation and in crop nitrate are found in the period when the sun passes the equator. In the northern hemisphere the nitrate content of lettuce decreases strongly in the period from March to May.

The effect of the amount of plant-available nitrogen in the soil on the nitrate content of the crop depends on global radiation (Table 2). In the dark period the nitrate content of the soil has little or no effect. Even at a low soil-nitrogen level the nitrate content is about 4000 mg NO_3 kg^{-1} fresh lettuce. In summer with high radiation levels the effect of soil nitrogen-supply is more pronounced.

In lettuce samples collected all the year round, 60% of the variation in nitrate content could be accounted for by global radiation and soil nitrate content.

TABLE 2

The Influence, Per Period of Two Months, of the Nitrate Content of the Soil (x, mmol NO_3 litre^{-1} extract) on the Nitrate Content in Lettuce (y, mg NO_3 kg^{-1} fresh wt.)

Period	*Regression equation*	*Corr. coeff.*	$\bar{x}$
June–July	$y=134x+1\,675$	0·48	1·7
Aug.–Sept.	$y=106x+2\,250$	0·25	2·3
Oct.–Nov.	$y=-17x+3\,475$	−0·06	3·8
Dec.–Jan.	$y=10x+3\,628$	0·06	4·5
Feb.–March	$y=57x+3\,313$	0·25	4·0
Apr.–May	$y=235x+1\,869$	0·64	2·3
Total	$y=158x+2\,451$	0·44	3·7

Methods to reduce the nitrate content of lettuce were discussed. The most attractive method is breeding varieties with low nitrate accumulation. Application of nitrification inhibitors is possible, but is as yet attended by too many drawbacks. By growing lettuce by nutrient film technique its nitrate content can be lowered by about 20%, but this method of cultivation is not yet economically feasible.

The Usefulness of Expressing Nitrogen Concentrations in Crops on the Basis of Tissue Water

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Concentrations of total nitrogen in plant tissues are often expressed on a dry matter basis and, generally, they decline as the plants develop.[1] Potassium concentrations in dry matter behave in a similar way but, in this case, the decline was found to result from changes in tissue hydration and could be eliminated by recalculating concentrations on the basis of tissue water.[2] Potassium concentrations expressed on the basis of tissue water were more constant with time, were responsive only to changes in potassium supply and appeared to be more physiologically relevant than concentrations in dry matter.[2] The possible advantages of expressing nitrogen concentrations in this way have been examined.[3]

Samples of spring barley (*Hordeum vulgare* cv. Georgie or Triumph) were collected every 3–5 days from experimental plots at Rothamsted and Woburn Experimental Farm, Bedfordshire. The plots received adequate phosphorus and potassium but were given different nitrogen treatments which resulted in differences in crop growth and grain yield. Sampling began when the plants had three leaves and continued until harvest. On each sampling occasion, all aerial plant parts were taken from a 1 m length of row, sealed in polythene bags, and brought to the laboratory where fresh and dry weights and nutrient contents were determined. Tissue water was assumed to be the difference between fresh and dry weight. Total nitrogen (including nitrate) was determined by Kjeldahl digestion.

Young barley shoots had nitrogen concentrations of 8–10 g kg^{-1} tissue water which declined to about 4 g kg^{-1} by anthesis. Thereafter, the concentration in the shoots increased and the rate of increase became more rapid during senescence. There were no differences in the values or behaviour of nitrogen concentrations in tissue water in crops grown with no nitrogen or those grown with 144 kg N ha^{-1}, even though the lack of nitrogen inhibited growth. Leaves had higher nitrogen concentrations in tissue water than stems and the decline in concentrations to anthesis was due to increases in the amount of stem tissue. The post-anthesis increase in concentrations was due to the development of the ears which had higher concentrations than other tissues.

Although lack of nitrogen decreased growth, it had no effect on nitrogen concentrations. The simplest interpretation is that tissues must have a certain nitrogen concentration and that decreases in nitrogen supply limit the amount of tissue that can be produced with this nitrogen concentration. Nitrogen concentrations in tissue water may, therefore, provide a physiological link between nitrogen supply and growth. The results also indicate that, at any particular stage of growth, the total amount of nitrogen in a crop must be directly proportional to the amount of water. As water contributes most of the fresh weight of a plant, fresh

weight may provide an estimate of the total nitrogen content of a crop and this may be of practical benefit when determining nitrogen use by crops.

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Biochemical and Molecular Genetics of Nitrogen Assimilation and Re-assimilation in Barley

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Photorespiration, the light- and O_2-dependent release of CO_2, has been identified as a major limitation to photosynthetic efficiency (and hence ultimate yield) in plants. This process also involves a release and re-fixation of ammonia, at a rate many times that of primary N assimilation. By selecting for barley mutants that are unable to survive in air, we are able to pin-point the enzymes involved in this N assimilation and to study their physiological role and genetics.

Three classes of mutants have been identified with reduced ability to reassimilate photorespiratory N. The first class is deficient in chloroplast glutamine synthetase (GS). This enzyme, present as two isoforms in barley leaves, catalyses the first step in NH_3 assimilation. Eight lines have been selected, and genetic analysis shows that the mutation is in a single nuclear gene. These mutants have no detectable chloroplast enzyme, but normal, wild-type, activity of the second isozyme. They are viable, healthy and fertile when grown in 0·8% CO_2, conditions that minimise photorespiration. In air, their rate of CO_2 fixation declines, accompanied by a massive rise in free NH_3 concentration in the leaves.

The second class of mutants have no detectable glutamate synthase (ferredoxin dependent). Again, in 0·8% CO_2 these plants (eight lines) are healthy and fertile. Genetic analysis demonstrates that all the lines are allelic, and the lesion is due to mutation of a single nuclear gene. The physiological characteristics of these

plants differ from those lacking GS in that CO_2-fixation in air drops rapidly, with a much lower build-up of NH_3.

The third class of mutants (three lines) have defective uptake of 2-oxoglutarate (substrate for glutamate synthase) into the chloroplast. They behave similarly to the second class of mutants, but are less sensitive to damage in air. A single nuclear gene is the site of the mutation. These mutants offer conclusive proof that glutamate synthase is restricted to the chloroplast.

The overall conclusion to be drawn from these studies is that photorespiratory NH_3 is assimilated via the 'glutamate synthase cycle', and that the major isoforms of these enzymes are not essential for growth and development when photorespiration is suppressed. Further study of these mutants will be invaluable in understanding the biochemistry and physiology of photorespiration, and in the detailed study of the genes for the key enzymes of N assimilation. Such work will have significance for herbicide design and herbicide resistance in crop plants.

Nitrate Assimilation in the Potato Crop

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N is an important determinant of crop yield, affecting many physiological processes. The three most important processes affected can be considered to be (1) leaf expansion, (2) carbon partitioning and (3) senescence. Once nitrate has been taken up by the plant the rate limiting step in the assimilation process is considered by many to be catalysed by the enzyme nitrate reductase (NR). The development of leaf NR activity has been followed in potato plants grown in the field under a range of N regimes.

The youngest expanded leaves from the top of the canopy were analysed for NR activity (*in vivo* assay), nitrate, protein and amino acid. The nitrate and amino acid contents of xylem sap from the petioles supplying these leaves were determined after exuding sap with a pressure bomb. Whole plant nitrate assimilation was determined following the analysis of nitrate-N and total N contents of leaves, stems and tubers.

NR activity (nitrate included in assay medium) increased to a peak at about 90 days after planting and declined thereafter. The decline was not prevented by maintaining a high N supply to plants. Data obtained from a comparison of NR activity with and without nitrate in the assay medium suggested that nitrate reduction may be well below potential for most of the season. However, nitrate reduction in fertilised plants may have constrained protein synthesis in the earliest stages of canopy expansion when low NR activity coincided with high concentra-

tions of nitrate in leaf and xylem sap. This may, in part, account for the fact that up to 25% of total plant N in fertilised plants was in the form of nitrate during this period. The low levels of daily solar radiation which accompanied low NR activity at this time may also have contributed to the high percentage of nitrate-N, by limiting the supply of reductant for NR. Nitrate-N stored in leaves and stems was assimilated in the latter part of the season and together with the mobilisation of protein reserves maintained tuber growth under conditions of reduced soil nitrate supply.

Under conditions of standard fertiliser practice, where all of the N is applied pre-planting, NR activity is likely to limit growth of the potato crop only during the early period of canopy expansion. The supply of soil nitrate and the release of nitrate previously stored in leaves and stems appears to limit assimilation for the greater part of the growth season. If NR activity does constrain canopy expansion then increasing the quantity of NR protein, and/or its affinity for nitrate, may result in earlier canopy closure and an extended period of light interception. The effects of substantial increases in the rate of nitrate reduction alone on yield and harvest index have yet to be determined, as has the influence of such manipulation on other processes dependent upon nitrate, e.g. osmoregulation.

Nitrosamines in Foodstuffs and Crops

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The occurrence of *N*-nitroso compounds in foodstuffs is of concern due to the biological activity many of these compounds exhibit in animal feeding studies. The concentration of the volatile compound *N*-nitrosodimethylamine (NDMA) in commodities such as cured meats and beer is now well established. Far less is known about the identity or levels of non-volatile *N*-nitroso compounds in foods or the factors that affect their formation. The occurrence of these compounds has been examined in a range of foodstuffs and raw materials that are potentially subject to nitrosating conditions during production or processing.

The concentration of apparent total *N*-nitroso compounds (ATNC) was measured by direct chemical denitrosation of the food and chemiluminescence detection of the released nitric oxide. This group-selective technique provides an estimate of all the *N*-nitroso compounds in the sample but gives no information on the concentration of individual species. Volatile and known non-volatile *N*-nitroso compounds were determined chromatographically.[1]

The concentrations of ATNC were below 50 μg (*N*-NO) kg^{-1} in a number of foodstuffs including biscuits, dried soups, coffee powder, milk powder, drinking

chocolate and tea leaves.[2] Brewing malts contained variable amounts of ATNC in levels of up to 400 μg (*N*-NO) kg^{-1}. The presence of these compounds in malt arises from the reaction of nitrogen oxides (NO_x) present in the drying gases that are used during kilning of germinated barley with amines on the exterior of the grain. The formation of NDMA during kilning can be substantially inhibited by the introduction of sulphur dioxide into the drying gases. However, recent studies have shown that the formation of the large majority of *N*-nitroso compounds is unaffected by this treatment and that ATNC can only be significantly reduced by minimizing the NO_x content of the drying gas.[3] This may be achieved using indirect fired or low-NO_x gas-fired kilns. On-going investigations into ATNC levels in beer have revealed that for some samples concentrations are higher than would be anticipated from the amounts present in malt. Subsequent studies[4] have shown that ATNC can also be formed during fermentation. This phenomenon appears to result from the presence of low levels of microbial species possessing nitrate reductase activity in the fermenting wort. Studies are currently in progress to establish whether ATNC are present in other fermented beverages and foodstuffs including bread, cheese and yoghurts. We have recently observed that silage may contain ATNC concentrations of up to 1–2 mg (*N*-NO) kg^{-1}. It seems likely that this also results from microbial nitrate reduction. Cured meats are also known[2] to contain ATNC in amounts of up to 1–2 mg (*N*-NO) kg^{-1} and this arises from the use of nitrite as a curing agent. Smoked cured meats contain up to 1 mg kg^{-1} concentrations of certain specific *N*-nitroso derivatives of heterocyclic carboxylic acids.[1,5] These arise from the reaction of woodsmoke aldehydes with cysteine followed by nitrosation of the heterocyclic product.

Comparison of the concentrations of ATNC in malts, beers and cured meats with the levels of known *N*-nitroso compounds in these commodities indicates that the large proportion of ATNC are of unknown identity. Further work is required to identify these compounds and establish the factors affecting their formation. In general the level of NDMA in foods does not provide even a qualitative indication of ATNC.

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Effect of N Level on the N Content of Tomatoes

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Single-truss tomato plants were grown in sub-irrigated troughs filled with a soil/peat/grit (7:3:2) mixture. Responses to N were exaggerated by the sub-irrigation because water was lost mainly via the plants and the amounts of nutrient solution used depended on growth, i.e. on N level. Some responses are shown in Table 1.

Growth and yield were best related to the weight rather than the concentration of N applied. In the fruit, a high proportion (80–90%) of the N was present as α-amino N and very little as NO_3-N (Table 2). Although NO_3-N was not determined in the leaves, past experience suggests that those grown with 250 mg NO_3-N litre^{-1} in the feed would have contained at least 0·4% as NO_3-N. Nevertheless, very little NO_3-N was imported into the fruit.

TABLE 1
Effect of N Level on the Growth and Yield, and on the Uptake and Distribution of N in Single-Truss Tomato Plants

N in feed (mg litre^{-1})	*Leaf length (cm)*	*Yield of fruit (g plant^{-1})*	*N applied (g plant^{-1})*	*Total N uptake (g plant^{-1})*	*% Total uptake in fruit*
100	16	111	0·90	0·25	59
150	26	279	1·91	0·91	53
200	31	382	3·19	1·78	53
250	35	433	4·40	1·86	54

TABLE 2
Effect of N Level on Some Forms of N in Tomato Fruit and on the N Content (Kjeldahl) of the Leaves

N applied (g plant^{-1})	*N in expressed fruit juices (mg 100 ml^{-1})*				*N content of leaves (%)*
	Total N	*α-amino N*	*NH_4-N*	*NO_3-N*	
0·90	48	38	1·1	0·06	2·36
1·91	81	66	1·9	0·10	3·00
3·19	104	92	2·7	0·22	3·90
4·40	136	120	4·1	0·29	4·88

Conclusion

The data show that tomato fruit contain low levels of NO_3-N, even when the crop is grown at a high level of N.

Fertilizer Practice, Nitrate-N and Organic-N in Vegetables

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The object of the work was to find the effect of N-fertilizer level on the nitrate and organic-N contents, at the usual time of harvest, of 14 different crops.

Each crop was grown in two single year experiments. All experiments were on separate sites within the same field. Until used for experiments, the sites were cropped continuously with cereals. Therefore, they all had an ADAS index of 0.

Nitrate-N concentrations were always less than the limit of detection (50 μg g^{-1} of dry matter) in the seeds of broad bean, French beans and peas; in the storage roots of carrots, parsnips and sugar beet, in the bulbs of onions and in the whole plants of leeks, despite the application of up to at least 315 kg N ha^{-1}.

Nitrate-N concentrations in the storage roots of red beet, swede and white turnip were between 140 and 870 μg g^{-1} dry matter with the optimum levels of fertilizer-N and between 3000 and 6900 μg g^{-1} of dry matter when very high levels were applied. The corresponding ranges of NO_3-N for the foliage of lettuce, spinach, summer cabbage and winter cabbage were 3000 and 4000 and 3600 and 7200 μg g^{-1} of dry matter.

From these results, and the surveys of fertilizer practice and food consumption in the UK, we estimate the average per capita dietary intake of nitrate in vegetables as 64 mg NO_3-N $week^{-1}$. This figure compares with a weekly intake of 24 mg NO_3-N in water. We also estimate that cabbage and lettuce contribute about 75% of total dietary nitrate in vegetables, a factor which may be useful for epidemiological studies on the effect, if any, between dietary nitrate intake and the incidence of stomach cancer.

For storage roots of red beet swede and white turnips, the % organic-N was always linearly related to % NO_3-N in the dry matter. The equation of best fit was:

$$\%\ \text{organic-N} = C + 4{\cdot}79\ (\%\ NO_3\text{-N})$$

where $C = 0{\cdot}15$ for red beet, $0{\cdot}19$ for swede and $0{\cdot}08$ for white turnip. The model removed 89% of the total sum of squares.

A general relationship was also found between functions of % organic-N and % NO_3-N that covered both foliage and root crops.

Suppose FN is the % organic-N of a crop grown with a given level of fertilizer expressed as a fraction of the % organic-N of the crop when grown with the optimum level. Also suppose FNO_3 is the % NO_3-N when grown with a given level of N-fertilizer expressed as a fraction of the % NO_3-N with the optimum level of N fertilizer then

$$\ln (FN) = K \ln (FNO_3) + D$$

where K and D are coefficients. With $K=0{\cdot}24$ and $D=0{\cdot}112$ for swede and $K=0{\cdot}21$ and $D=0{\cdot}0331$ for all other crops, the model removed 87·5% of the total sum of squares.

These relationships emphasise the dependence of % organic-N on the nitrate contents of the crops but the reasons for them are not clear.

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Protein and Enzyme Content of Lettuce Grown in the Presence of Nitrogen Oxides

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The concentration of CO_2 in the atmosphere limits the rate of photosynthesis and in glasshouses raising the CO_2 level enhances photosynthesis, growth and yield. Crops can be grown economically by using direct-fired natural gas burners which provide both CO_2 and warm-air heating. However, NO and NO_2 (NO_x) gases are also formed and these can be detrimental to the plants, reducing the rate of photosynthesis. The mechanisms by which NO_x gases reduce CO_2 fixation have yet to be determined. The activity of a number of enzymes involved in nitrogen metabolism including nitrite reductase (NiR) is increased in certain cultivars of tomato when exposed to low levels of NO.[1] Wellburn and colleagues[1] suggested that these increases could be a detoxifying mechanism and that plants may benefit from the additional source of nitrogen. While NiR activity frequently increases in NO_x-treated plants the response is variable and depends, for example, on species, variety, irradiance and NO_x concentration. In this work, data on enzymes involved in C and N metabolism are presented, using the lettuce cultivars Pascal and Talent which in preliminary trials showed greater tolerance to NO_x than some other cultivars.

Lettuce were grown in glasshouses where direct-fired natural gas burners were used for heating and to provide CO_2 enrichment (high CO_2+NO_x treatment) and control plants were grown in glasshouses enriched with pure CO_2 and heated with a piped hot-water system. A segment was cut through each lettuce so that leaves of all ages were included and proteins were extracted as previously described.[2,3] Extracts were heated in boiling water for 2 min in 2% sodium dodecyl sulphate

(SDS), 10% glycerol, 1% 2-mercaptoethanol, 50 mM Tris–HCl, pH 7·4 before electrophoresis. Antibodies to NiR were raised in rabbits. Electrophoresis of proteins was performed on a discontinuous gradient of SDS-polyacrylamide consisting of a 10% acrylamide running gel and a 3% stacking gel. After SDS-polyacrylamide gel electrophoresis (PAGE), proteins were electrophoretically transferred (Trans Blot Cel, BioRad Laboratories) on to nitrocellulose sheets (Schleicher and Schuell, BA83) using a Tris–glycine–methanol buffer, pH 8·3. Following electrophoretic transfer the nitrocellulose was incubated overnight at 4°C or for 1 h at room temperature in a blocking solution consisting of 3% (w/v) bovine serum albumin (BSA) in TBS (50 mM Tris–HCl, pH 7·4 and 200 mM NaCl), then incubated with antibodies to NiR (1/500 dilution) in TBS+1% BSA for 2 h at room temperature. A 1/500 dilution of preimmune serum was used as a control. The nitrocellulose was washed for 30 min with four changes of TBS containing 0·1% (w/v) BSA and 0·05% (w/v) Tween 20. The second antibody, peroxidase-conjugated goat-antibodies to rabbit IgG (0·1 mg ml^{-1}) was diluted 1/1000 in TBS, 1% BSA and 1% goat IgG and incubated with the nitrocellulose for 1·5 h. Then the nitrocellulose was washed for 30 min with four changes of TBS containing 0·1% (w/v) BSA and 0·05% (w/v) Tween 20. The peroxidase activity was revealed by incubating the nitrocellulose with 3 mg ml^{-1} 4-chloro-1-naphthol and 0·015% H_2O_2 in TBS.

On a fresh weight basis the activities of the chloroplast enzymes involved in C metabolism RuBP carboxylase, 3PGA phosphokinase, and glyceraldehyde 3P dehydrogenase were all higher in extracts from the cultivar Pascal grown in the (high CO_2+NO_x) treatment. The cytoplasmic enzyme PEP carboxylase was also significantly increased. The activity of the chloroplast enzyme NiR was also significantly increased by the (high CO_2+NO_x) treatment. However, neither the soluble protein nor Kjeldhal nitrogen in the leaves was increased on a fresh weight basis by the (high CO_2+NO_x) treatment but both were significantly higher on a per plant basis. Similar increases in activity of NiR with the cultivar Talent were observed after prolonged exposure to the high CO_2+NO_x gases. The amount of NiR protein was also higher in leaf extracts from the (high CO_2+NO_x) treatment. As revealed by SDS-PAGE immunoblotting, an increased amount of single molecular species was present with an apparent mol. wt in SDS of approximately 62 000.

Although the immunoblotting technique provides only semi-quantitative information, the increase in NiR activity observed in the cultivar Talent is accompanied by an increase in the steady-state concentration of NiR protein. In addition, in these preparations no additional bands of NiR protein appeared, suggesting that the induced NiR activity was the result of increased levels of the same molecular form of NiR.

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Environmental Factors Influencing Nitrate Reductase Activity in Winter Barley

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Currently, there are no simple soil or crop tests which can accurately predict fertiliser-N requirements and timing thereby improving fertiliser-N recovery by the crop. The objective of this work was to investigate the manner in which nitrate reductase (NR), a substrate-induced enzyme, is influenced by environmental factors (including N nutrition), with a view to exploring the use of its activity as an indicator of crop-N status.

Winter barley plants (cv. Igri) were grown in a recirculating nutrient solution culture system which allowed air and root temperature, pH, light intensity and nutrient composition to be controlled. Plants were harvested at the 4th leaf stage and fresh and dry weights of shoots (but not roots) recorded. Fresh shoots and roots were assayed[1] for endogenous (NRA_e) and induced (NRA_i) nitrate reductase activity. Dried samples were finely milled and analysed for reduced-N and NO_3-N.

When constant N (0·57 mM NO_3-N) and temperature (10°C) were maintained at the root, significantly ($P<0·05$) more NO_3-N was absorbed and reduced at an air temperature of 17°C than 6°C. NRA_e and NRA_i were significantly ($P<0·05$) higher in plants grown at 6°C compared with 17°C, indicating that low air temperatures appear to favour synthesis/activation of NR.[2] Plants grown with constant-N (0·57 mM NO_3-N) and an air temperature of 10°C, but at root temperatures of 5, 10 and 15°C showed root temperature to have a similar effect on total-N, NO_3-N, NRA_e and NRA_i as air temperature. Nitrate assimilation capacity, NAC (i.e. NRA_i/NRA_e), was constant at all air and root temperatures.

In another experiment plants were grown at a constant 10°C air and root temperature and in complete nutrient solutions maintained at pH 4·0, 5·3 and 6·5 containing 0·57 mM NO_3-N. Shoot and root NO_3-N, NRA_e and NRA_i decreased significantly ($P<0·05$) with increasing pH due to greater uptake of NO_3-N and NR induction. NAC in roots or shoots was unaffected by pH.

When plants were grown in complete nutrient solutions varying only in NO_3-N (i.e. 0–4·57 mM NO_3-N) and with an air and root temperature of 10°C, NRA_e and NRA_i increased from negligible values with zero NO_3-N to maxima at >1·14 mM NO_3-N. Nitrate uptake rates from solutions containing 0·14 and 0·29 mM NO_3-N were insufficient to prevent severe and slight N deficiency symptoms respectively in these two treatments.

In the final experiment, plants were grown at 10°C air and root temperature in complete nutrient solutions containing 1·43 mM N either as 100% NH_4-N, 100% NO_3-N or 50% NH_4-N+50% NO_3-N. Significantly ($P<0·05$) higher total-N and shoot yield were found for the 100% and 50% NH_4-N treatments compared to the 100% NO_3-N treatment. Negligible NRA was observed in plants receiving 100% NH_4-N nutrition. There was significantly ($P<0·05$) greater NRA_e and NRA_i in shoots grown in the 100% compared to the 50% NO_3-N solution. However, similar NAC quotients for all treatments indicated a high plant N status.

The main conclusion arising from this work is that nitrate assimilation capacity is a sensitive indicator of the N status of winter barley plants irrespective of form and concentration of N, pH and temperature.

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The Effect of Fertilizer Application on the Nitrogen Uptake and Content in Grass in Cereals

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Three years of fully replicated experiments have been carried out at Norsk Hydro Demonstration Centres throughout England and Wales from 1983 to 1985.

Grass

The dry matter (DM) yield response to nitrogen fertilizer has been assessed at 19 sites on predominantly Italian ryegrass/perennial ryegrass swards. The trials show that the economic optimum nitrogen rate, assuming nitrogen fertilizer at 40p kg^{-1} and grass dry matter at £60 t^{-1}, was 143 kg ha^{-1} for first cut silage and 104 kg ha^{-1} for second cut silage.[1]

The nitrogen content and nitrogen uptake continued to increase beyond the economic optimum rates for dry matter production. At a given nitrogen rate the nitrogen content of second cut silage was much higher than that for first cut (Table 1).

Thus the protein content of first cut silage could be increased by applying more nitrogen without obtaining excessively high nitrogen contents. This gain in crude protein content would have to be offset against the loss in dry matter yield. Whereas higher than optimum rates for second cut silage would give problems of poor fermentation and consequent inefficient utilisation of the silage in the animal rumen.

TABLE 1
The Effect of Nitrogen on Yield, Nitrogen Content and Nitrogen Yield of First and Second Cut Silage

First cut silage				*Second cut silage*			
Nitrogen rate (kg ha^{-1})	*DM yield (t ha^{-1})*	*Nitrogen content (% DM)*	*Nitrogen uptake (kg ha^{-1})*	*Nitrogen rate (kg ha^{-1})*	*DM yield (t ha^{-1})*	*Nitrogen content (% DM)*	*Nitrogen uptake (kg ha^{-1})*
0	3·32	1·25	42	0	2·36	1·21	29
75	5·76	1·41	81	50	3·55	1·63	58
150	6·70	1·84	123	100	4·17	1·94	81
225	6·46	2·20	142	150	4·27	2·34	100

Winter Wheat

The grain yield response to nitrogen fertilizer has been assessed in 28 trials on a range of varieties of winter wheat. The trials show that the economic optimum nitrogen rate, assuming nitrogen fertilizer at 40p kg^{-1} nitrogen and grain at £100 t^{-1}, was 186 kg N ha^{-1}.

The grain protein content continued to increase beyond the optimum rate for grain yield. The response to nitrogen varied with variety. The cultivar Avalon showed a greater increase in protein content with increasing nitrogen rate than the cultivar Longbow.

Thus the protein content of winter wheat can be increased by the application of high rates of nitrogen fertilizer. However, any gain in protein content would have to offset the additional fertilizer cost. The use of excessively high rates of nitrogen may be economically and environmentally undesirable. Other trials examining late applications of nitrogen at growth stage 32 and ear emergence have shown no increase in protein content above that from a single application in mid April. Where high protein levels are desirable choice of variety may be important to gain the maximum return from nitrogen fertilizer.

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Gaseous Ammonia as a Source of Nitrogen for Grass

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Plants have been shown to take up ammonia (NH_3) through leaf surfaces, producing a growth response when the supply of nitrogen (N) to the roots is inadequate.[1] Substantial amounts of NH_3 are volatilised from grazed pastures and from fields treated with animal slurries or manures.[2] Some of this NH_3 is likely to be absorbed by plant leaves but little information is available on the factors affecting absorption.[3] The aim of the present study was to assess the influence of the concentration of NH_3 on its uptake by Italian ryegrass grown in soil at two rates of added nitrate.

Plants were exposed in a system of chambers[4] to nine concentrations of NH_3 ranging from 14 to 709 μg m^{-3}. Two rates of ^{15}N-labelled nitrate were supplied to the pots of soil in which the grass was grown, providing plants with either adequate or less than adequate N for maximum growth. After 33 days of exposure to NH_3 (16 h per day, corresponding to the photo-period) shoots were harvested at *ca.* 2·0 cm, the stubble was removed at soil level and the roots were recovered by washing free of the soil. Plan areas of the leaf and sheath components of the shoots were measured, together with dry weight, % N, and % ^{15}N in all plant fractions. Control plants that received no NH_3 or nitrate were used to assess the small amount of N in the plant derived from the soil, which was a sub-soil. Nitrogen derived from the added nitrate was calculated from the ^{15}N enrichment in each plant fraction. The amount of NH_3-N taken up from the atmosphere was calculated by subtracting the amounts derived from the soil and added nitrate from the total plant N.

The amount of NH_3-N taken up increased with increasing concentration of NH_3, the relationship being essentially linear but with a significant quadratic component:

$$\text{uptake (mg pot}^{-1}) = 0{\cdot}360x + 0{\cdot}000158x^2$$

where $x = \mu$g NH_3 m^{-3}. There was no difference in this relationship between the two rates of added nitrate. When the absorption of NH_3 was expressed in terms of uptake per unit leaf area per unit time, the relationship was linear and differed between the two rates of nitrate:

$$\text{uptake } (\mu\text{g dm}^{-2}\text{ h}^{-1}) = 0{\cdot}1009x \text{ (at low nitrate)}$$

$$\text{uptake } (\mu\text{g dm}^{-2}\text{ h}^{-1}) = 0{\cdot}0829x \text{ (at high nitrate)}$$

Regression analysis of the values for uptake of added nitrate showed a small but significant reduction in uptake with increasing concentration of NH_3 in the air. NH_3 contributed 4% of the total plant N at a concentration of 14 μg NH_3 m^{-3} with high nitrate and 77% of the plant N at a concentration of 709 μg NH_3 m^{-3} and low nitrate.

The results confirm that grass has the potential to derive substantial amounts of N from gaseous NH_3 and, considered in conjunction with indirect assessments of concentrations of NH_3 in the atmosphere within the canopy of grazed grass swards, suggest that this source may sometimes contribute up to 15–20% of the plant N.

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Varietal Variation and Correlation of Trace Metal Levels with 'Catechins' and Caffeine in Sri Lanka Tea

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ABSTRACT

Trace elements, phosphorus, 'catechins' and caffeine levels in different varieties of Sri Lanka tea have been determined. The range and mean of zinc, copper, iron, lead and cadmium in 73 tea samples were as follows: zinc 22·5–62·5 and 35·0 μg g^{-1}, copper 10·0–25·0 and 17·8 μg g^{-1}, iron 40·0–185·0 and 128·1 μg g^{-1}, lead 0·188–0·561 and 0·426 μg g^{-1}, cadmium 0·156–0·380 and 0·239 μg g^{-1}. Significant differences in zinc, iron, lead and cadmium were observed among tea varieties grown at different elevations in Sri Lanka. These levels were also compared with results obtained with samples from other countries. The phosphorus content in 'black tea' showed correlations with trace metal levels in the order lead>zinc>iron>copper>cadmium. Positive correlation with levels of 'catechins' were more significant with zinc and cadmium. Caffeine levels were found to be significantly correlated with lead and zinc in 'black tea'. Studies of translocation of trace metals from soil have involved analysis of various parts of the tea shoot and supporting soil.

Key words: Trace metals, catechins, caffeine, phosphorus, black tea.

1 INTRODUCTION

Tea is an important export commodity of Sri Lanka. Several studies have been reported on trace metal levels in tea.[1,2] These results have been attributed to

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varietal variations, to different agricultural, climatic, seasonal and soil conditions, as well as to environmental contamination in specific instances.

The present paper reports variables that influence trace metal levels in tea, as well as their translocation in the plant. It includes varietal variation of trace metal levels, as well as their possible association with other tea leaf constituents as caffeine and 'catechins'. The results of analysis of 73 samples of 'black tea' are presented. Trace metal status of zinc, copper, nickel, cobalt and lead of tea 'flush', stem and mature leaf have also been determined and correlations with supporting soil and different parts of the tea shoot obtained.

2 EXPERIMENTAL

2.1 Samples

'Black tea' samples were obtained from the Tea Research Institute, Talawakella, Sri Lanka. Representative samples of soil and of 'flush', stem and mature leaf of the greenleaf, were collected from each of the sampling plots, one of which is shown in Fig. 1, situated at the experimental station, St. Coombs' estate, Talawakella, Sri Lanka. About ten 'cores' of soil were collected from each of these sample plots in a 'zig-zag' manner, as given in Fig. 1 (b). They were thoroughly mixed together after breaking any lumps. The mixed samples from each of the plots were spread into separate layers and small portions were taken at random from each layer and mixed separately, to give composite samples from each of these plots. These composite samples were transferred into clean labelled polyethylene bags, sealed in the field and transferred to the laboratory. The final weight of each of these composite samples was about 500 g. These samples were then spread on clean polyethylene sheets, separately, in the laboratory and dried in air for two days. The dried samples were sieved to a mesh size of −55, without grinding and collected in clean labelled polyethylene bags.

2.2 Analysis

Preliminary analysis of results showed that 'wet' digestion with a mixture of concentrated HNO_3 and concentrated H_2SO_4 best represented trace metal levels.

Tea samples (1 g) were kept overnight in acid washed 100 cm^3 'Pyrex' beakers, with 5 cm^3 of a 5:1 mixture of concentrated HNO_3 and concentrated H_2SO_4. The samples were digested with the addition of 0·5 cm^3 $HClO_4$, in a fumehood on a hot plate initially controlled at 110°C. The temperature of the hot plate was gradually increased to 210°C to expel nitric and perchloric acid fumes. This procedure was repeated if necessary, with the addition of more of the mixture of concentrated HNO_3 and $HClO_4$, until a clear colourless solution was obtained. The resulting solution was then heated finally around 250°C, to expel sulphuric acid fumes, taking care to avoid splashing. A moist residue was then obtained.

The moist residue was taken up in 10 cm^3 2 M HCl and evaporated on a water bath. The resulting residue was dissolved in warm 0·5 M HCl and made up to 25 cm^3 in 0·5 M HCl. The lead, zinc, copper, cadmium and iron contents of the sample solutions were then determined by atomic absorption spectrophotometry.

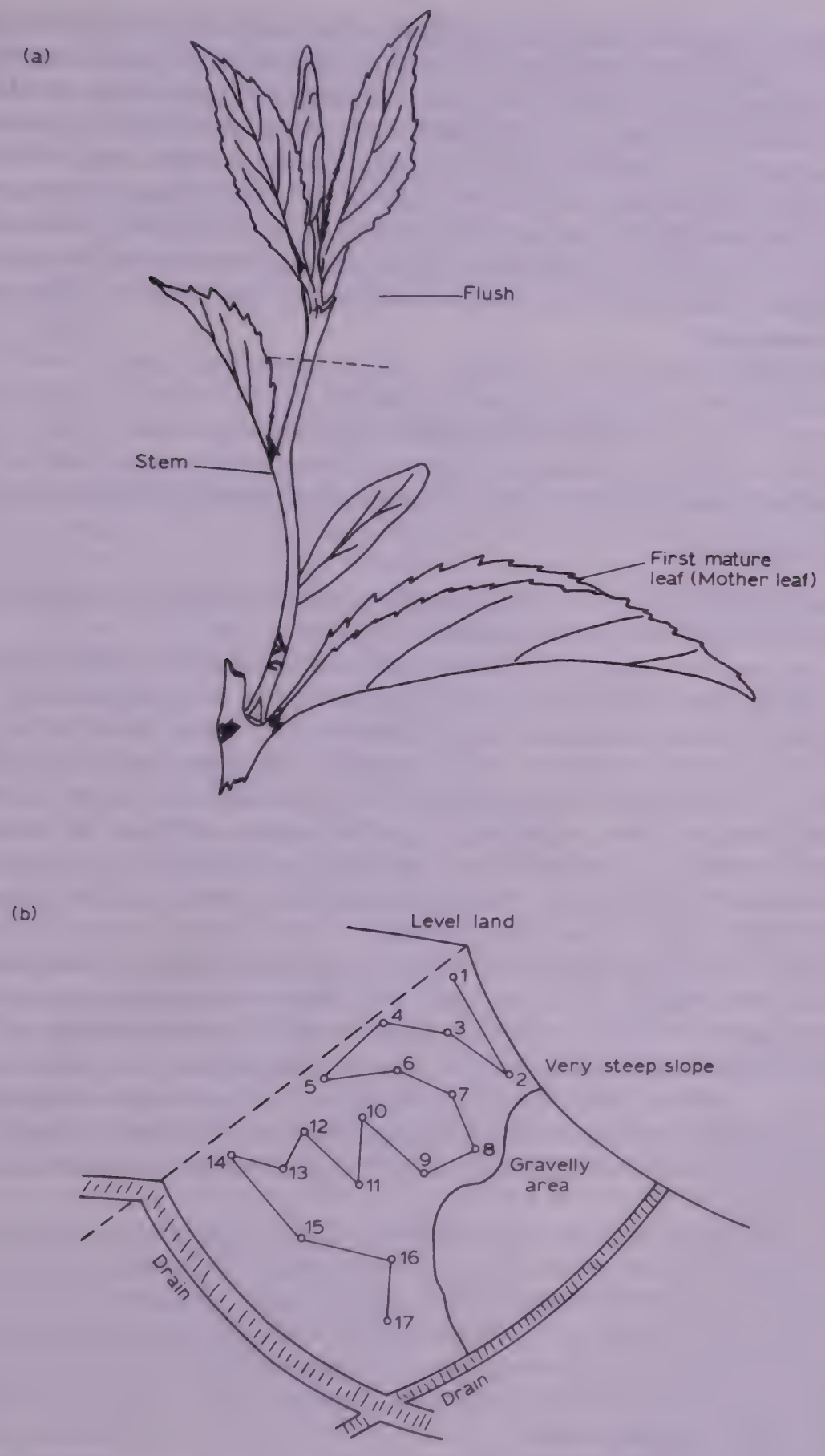

Fig. 1. (a) Sketch of tea shoot showing leaf to be sampled. (b) Sketch of soil sampling in an experimental plot to obtain a composite sample from that plot.

Preliminary studies showed that digestion of soil samples with a mixture of HF and $HClO_4$ was suitable for most trace metals. 2 cm^3 of $HClO_4$ and 5 cm^3 of HF were added to each of the 1 g portions of the prepared soil samples in 50 cm^3 'Teflon' beakers in triplicate and digested for 1 h on a water bath. The solution was evaporated to near dryness after adding a further 2 cm^3 each of HF and $HClO_4$. The moist residue was dissolved in 10 cm^3 2 M HCl and evaporated to dryness. The resulting residue was dissolved in 10 cm^3 0·5 M HCl, warmed and made up to 50 cm^3 in 0·5 M HCl. Zinc, copper, cobalt, nickel and lead contents of the sample solutions were then determined by atomic absorption spectrophotometry.

Exchangeable trace metal content of soil was measured using 0·1 M HCl as extractant for zinc, cobalt, nickel and lead and 0·1 M EDTA (pH 7) as extractant for copper.[3] 5 g of prepared soil samples were extracted with 25 cm^3 of the extractant for 1 h. The resulting solution was filtered and the filtrate analysed for zinc, copper, nickel, cobalt and lead by atomic absorption spectrophotometry.

2.3 Instrumentation

A Varian model A.A. 275, atomic absorption spectrophotometer, equipped with a laminar flow type acetylene air burner was used.

The analyses for copper, zinc, iron, nickel and cobalt were performed at the 324·7, 213·9, 248·3, 232·0 and 240·7 nm resonance lines respectively. The analyses for lead and cadmium were performed on a varian model A.A. 1275 atomic absorption spectrophotometer, equipped with a graphite tube atomizer assembly, with temperature programmer. These were carried out at the 217·0 and 228·8 nm resonance lines respectively. Sample volumes of 20 and 10 μl respectively were used for lead and cadmium, with ashing and atomising temperatures of 500°C and 1800°C respectively. The metal contents were determined by comparison with standards in the same acid media.

A Cecil C.E.273 spectrophotometer with matched glass cells was used to determine phosphorus contents using the molybdenum blue method with measurement at 830 nm.[4] A Waters associate HPLC with a solvent delivery system, incorporating model 6000 A isochromatographic pump (flow rate, 2 cm^3 min^{-1}, pressure 4000–4500 psi) and solvent programmer with gradient elution was used for determination of caffeine content of the samples. A pH of 3·5 by adjustment with glacial acetic acid and 30% aqueous methanol was used as the mobile phase.

1 g portions of tea samples were extracted with 25 cm^3 portions of chloroform and the extracts evaporated to dryness on a water bath. The residue was dissolved in distilled water, filtered and the filtrate together with washings was made up to 100 cm^3 in distilled water. 100 μl of the solution was injected into the h.p.l.c. and the caffeine content was determined by comparing with a series of standard solutions in the concentration range, 1–50 μl $litre^{-1}$, which was injected concurrently with the sample solution.

The water soluble constituents, 'catechins' ('catechins' refer to both original catechins, gallocatechins and their oxidised forms as thearubigens and theaflavins) of 'black tea' samples were estimated on a relative basis from the

absorbance values of ethanol extracts at 270 nm (absorbance at 270 nm of the extract, accounts for both the water soluble constituents, 'catechins', and caffeine in tea, the latter being determined independently using h.p.l.c. as described). Preliminary studies established use of ethanol as the extractant, and a particle size <mesh 60, as optimum conditions for the determination.

0·1 g portions of ground tea samples (mesh <60) were extracted with 25 cm^3 portions of ethanol by shaking for 5 min, filtered and solution made up to 50 cm^3 in ethanol. The absorbance at 270 nm was measured against a solvent blank. These absorbance values were used as a measure of the content of 'catechins' of these samples.

3 RESULTS AND DISCUSSION

Reproducibility of analyses was obtained using ten replicates of subsamples from a single sample of 'black tea'. Coefficients of variation for zinc, copper, lead, iron and cadmium in Sri Lankan 'up country' tea samples were 3·11, 3·28, 1·46, 1·99 and 3·64 respectively. The mean values obtained for these metals were 60·5, 25·0, 0·533, 61·0 and 0·220 $\mu g\ g^{-1}$ respectively. The standard deviation for these values were 1·88, 0·82, 0·007, 1·2 and 0·008 respectively.

Trace metal content of varieties of tea grown in different elevations in Sri Lanka, as well as those samples obtained from other countries are shown in Table 1. The zinc content among the different Sri Lanka varieties showed a coefficient of variation of 2·2. Sri Lanka, 'up country' tea showed a relatively high value of 62·5 $\mu g\ g^{-1}$, whereas those from 'mid country' and 'low country' were 22·5 $\mu g\ g^{-1}$, well below the overall mean of 35·01 $\mu g\ g^{-1}$. Among the foreign varieties, Malawi (clone 412) showed the highest value of 65 $\mu g\ g^{-1}$, while Indian (Dooars) variety had the lowest value of 21·3 $\mu g\ g^{-1}$. The copper content showed little variation between 'up country' and 'low country' varieties, whilst the 'mid country' variety showed a significantly low level. With foreign varieties, Indian (Assam) teas showed the highest level of 50 $\mu g\ g^{-1}$, while Malawi (clone 403) recorded the lowest value of 7·5 $\mu g\ g^{-1}$. The relatively low iron content of Sri Lanka tea compared with those from other countries is notable.

The contents of lead and cadmium in Sri Lanka teas were found to be different from those of International Standards Organisation data,[5] of a mean lead content of 0·09 $\mu g\ g^{-1}$. A high level of lead in 'up country' tea corresponded to a low level of cadmium, whereas the reverse trend was found with 'low country' tea.

A matrix of correlations between zinc, copper, iron and cadmium is given in Table 2. Zinc showed strong positive correlation with lead and cadmium. A negative correlation between lead and cadmium was found.

The results obtained for trace metal content in 'black tea' are subject to wide variations, not only among different varieties, but also within the same variety. These variations could be attributed to different agricultural, climatic, seasonal and soil conditions as well as on the maturity of the 'tea flush' on harvesting. The reports of Hasselo[6] and Tsushida *et al.*[7] indicate a decrease in trace metal levels in 'tea flush' with maturity.

TABLE 1
Zinc, Copper, Iron, Lead and Cadmium Contents in 'Black Tea' Samples Obtained from Different Elevations in Sri Lanka and from Other Countries

Producing region	*Number of samples*	*Zinc ($\mu g\ g^{-1}$)*		*Copper ($\mu g\ g^{-1}$)*		*Iron ($\mu g\ g^{-1}$)*	
		Range	*Mean*	*Range*	*Mean*	*Range*	*Mean*
Sri Lanka (up country)	12	51–70	62·5	22–27	25·0	34–48	40·0
Sri Lanka (mid country)	12	18–29	22·5	8–11	10·0	121–159	142·5
Sri Lanka (low country)	12	17–26	22·5	18–25	22·5	125–156	145·0
Sri Lanka (tea dust)	6	21–40	32·5	10–15	13·8	141–202	185·0
India ('Dooars')	6	16–25	21·3	23–26	25·0	116–127	120·0
India ('Assam')	6	22–27	25·0	41–56	50·0	221–271	250·0
India ('Darjeeling')	6	22–33	31·3	46–51	48·8	226–234	230·0
Malawi (clone 412)	3	60–71	65·0	32–33	32·5	270–286	280·0
Malawi (clone 403)	3	31–33	32·5	7–9	7·5	221–240	232·0
Kenya	3	32–33	32·5	36–39	37·5	291–297	295·0
China	4	33–37	35·0	16–18	17·5	253–236	255·0

Producing region	*Number of samples*	*Lead ($\mu g\ g^{-1}$)*		*Cadmium ($\mu g\ g^{-1}$)*	
		Range	*Mean*	*Range*	*Mean*
Sri Lanka (up country)	12	0·540–0·572	0·562	0·171–0·189	0·178
Sri Lanka (mid country)	12	0·467–0·482	0·415	0·141–0·190	0·156
Sri Lanka (low country)	12	0·121–0·241	0·188	0·152–0·269	0·241
Sri Lanka (tea dust)	6	0·402–0·521	0·482	0·379–0·396	0·388
India ('Dooars')	6	0·446–0·471	0·462	0–0·126	0·029
India ('Assam')	6	0·496–0·510	0·506	—	—
India ('Darjeeling')	6	0·409–0·479	0·456	—	—
Malawi (clone 412)	3	0·572–0·586	0·581	0·210–0·227	0·217
Malawi (clone 403)	3	0·469–0·481	0·477	0·096–0·127	0·115
Kenya	3	0·360–0·364	0·362	—	—
China	4	0·291–0·311	0·306	—	—

TABLE 2
Matrix of Correlation Between Trace Metal Levels in 'Black Tea' Varieties ($P \leq 0{\cdot}05$)

	Zinc	*Copper*	*Iron*	*Cadmium*
Copper	0·056			
Iron	0·360	0·349		
Cadmium	0·408	0·260	0·169	
Lead	0·529	0·127	0·147	−0·268

Thus the level of maturity at harvesting also accounts for the variations in trace metal levels in 'black tea'. As tea from young 'flush' is of higher quality as compared to 'aged flush', quality teas should contain higher levels of trace metals than poor quality tea, levels of trace metals such as lead and cadmium could depend more on effects of atmospheric pollution than from any growth factors. Tsushida *et al.*[7] report marked reduction in lead levels in tea 'flush' upon washing with water, prior to analysis. However, levels of cadmium were unaffected on washing.

The contents of phosphorus ($\mu g\ g^{-1}$) and caffeine (%) in 'black tea' varieties from different regions were determined and the results are shown in Table 3.

Preliminary studies showed that the absorbance of an ethanol extract of 'black tea' at 270 nm was an index of the content of 'catechins'.

TABLE 3
Phosphorus ($\mu g\ g^{-1}$) and Caffeine (%) Contents in 'Black Tea' from Different Regions

Region or country of production	*Phosphorus ($\mu g\ g^{-1}$)*	*Caffeine (%)*
Sri Lanka (up country)	1019	2·88
Sri Lanka (mid country)	1002	2·02
Sri Lanka (low country)	872	1·79
India ('Dooars')	953	2·26
India ('Assam')	1064	1·23
India ('Darjeeling')	1267	2·92
Malawi (clone 412)	1302	3·07
Malawi (clone 403)	1117	2·42
Kenya	963	2·68
China	1060	1·34

Variation of absorbance at 270 nm of ethanol extracts of 'black tea' showed optimum extraction as compared with the use of water, *n*-butanol and chloroform as extractants. The absorbance was measured within the first 30 min as decreases were observed thereafter. The linear correlation coefficients between levels of phosphorus, 'catechins', and caffeine in 'black tea', with trace metal contents of 'black tea' from Sri Lanka (up country) are shown in Table 4.

The positive correlation of phosphorus content with trace metal levels were found to be in the order lead>zinc>iron>copper>cadmium. The significant correlations obtained may be attributed to the phosphorus carrying properties of trace metals in plants, as reported for zinc.[8] The high correlation with levels of lead and the very low correlation with cadmium are to be noted. All trace metal levels showed positive correlation with levels of 'catechins', those with zinc and cadmium being more significant. The caffeine levels were found to show significant correlation with lead and zinc content in 'black tea' samples grown in Sri Lanka ('up country').

The content of zinc, copper, nickel, cobalt and lead in 'flush', mature leaf and stem of the fresh leaf, as well as those in the supporting soil (both total and extractable) were determined. Trace metal contents were extracted from soils by means of 0·1 M HCl for zinc, cobalt, nickel and lead. EDTA, 0·1 M was the

TABLE 4
Linear Correlation Coefficients Between Trace Metal levels in 'Black Tea' Samples Grown in Sri Lanka 'Up Country', and Contents of Phosphorus, Relative Water Soluble 'Catechins' (as Indexed by Absorbance at 270 nm of Ethanol Extracts) and Caffeine ($P \leq 0{\cdot}05$)

Trace metal	*Phosphorus*	*'Catechins'*	*Caffeine*
Zinc	0·529	0·547	0·482
Copper	0·335	0·323	0·231
Iron	0·465	0·170	−0·243
Cadmium	0·142	0·472	−0·142
Lead	0·591	0·357	0·521

TABLE 5
Mean Values of Trace Metals in Different Parts of the Plant and Supporting Soil, Obtained Using 21 Samples in Each Case

Trace metal	*Plant ($\mu g\ g^{-1}$)*			*Soil ($\mu g\ g^{-1}$)*	
	Flush	*Mature leaf*	*Stem*	*Total*	*Extractable*
Zinc	29·7	17·6	25·62	67·6	1·49
Copper	16·94	12·43	18·25	39·3	9·51
Iron	6·8	10·7	1·9	67·2	0·203
Cadmium	0·674	1·32	1·08	42·5	1·13
Lead	0·482	0·990	0·855	5·3	1·80

extractant for copper.[3] These extractants and optimum conditions were established in preliminary studies. The results obtained for correlation with different parts of the tea shoot, extractable and total trace metal of supporting soil are found useful to indicate trace metal content in the 'flush' used in 'black tea'.

The mean values of the trace metals in different parts of the tea shoot and supporting soil are given in Table 5.

The zinc content in different parts of the tea shoot were found to be in the order, 'flush'>stem>mature leaf. The zinc content in stem was shown to be significantly correlated to that in mature leaf with a high correlation coefficient of 0·895.

However, the zinc content in 'flush' did not show any significant correlation with its content in mature leaf and hence the latter could not be used as an indicator of zinc content in 'flush'. No significant correlations were found between the zinc content of soil and various parts of the tea shoot.

The copper content showed an order, stem>'flush'>mature leaf. A significant correlation between copper content in 'flush' and mature leaf enables the latter to be used as an index for the copper status in flush. As with zinc, no significant correlations are found between copper status in soil and plant.

The nickel content in different parts of the plants was in the order, mature leaf>'flush'>stem. Significant correlations between nickel content in mature leaf and stem to that in 'flush' (0·618 and 0·747 respectively) permits both mature leaf and stem to be used as indices of the nickel content of 'flush'. The nickel content in all parts of the tea shoot was found to be linearly correlated with extractable nickel content in soil. The linear correlation coefficient of extractable nickel content with that in 'flush', mature leaf and stem were 0·767, 0·634 and 0·624, respectively.

The cobalt content in different parts of the tea shoot was found to be in the order, mature leaf>stem>'flush'. Although the cobalt content in 'flush' was significantly correlated with that in stem (correlation coefficient=0·514), no such correlation was obtainable with that in mature leaf. The mature leaf does not represent the 'flush', as regards its cobalt content. The extractable content of cobalt in supporting soil showed a significant correlation only with that in 'flush' (correlation coefficient=0·614).

The lead content in tea shoot was in the order mature leaf>stem>'flush'. Its content in 'flush' significantly correlated with that in stem and a poor correlation

was obtained with that in mature leaf. The correlation coefficients being 0·746 and 0·366 respectively. The extractable lead content showed very significant correlation only with that in 'flush'.

These results show that the relative distribution of trace metals in 'flush', stem and mature leaf depend on the trace metal. Zinc levels were highest in 'flush', whereas cobalt, nickel and lead contents were higher in mature leaf. Copper contents were found to be highest in the stem. A significant feature observed in this study was the high correlations between extractable lead, cobalt and nickel of soil and tea shoot. These were found to be more significant than the correlations obtained for the biologically active zinc and copper content.

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Determination of Moisture in White Flour, Ground Wheat and Whole Wheat by Near Infrared Reflectance Using a Single Calibration

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ABSTRACT

A single calibration for the determination of moisture in white flour, ground wheat and whole wheat by near infrared (n.i.r.) reflectance analysis is presented. The predictive accuracy of the calibration compared with moisture determined by oven drying on 68 samples was 0·22%.

Key words: Moisture, near infrared reflectance, flour, wheat, wavelength selection.

1 INTRODUCTION

Near infrared (n.i.r.) reflectance spectroscopy[1] has been developed over the past ten years into a widely accepted method of food analysis. However, it is regarded as a technique which requires sample-specific calibrations and hence is limited to situations such as grain trading where large numbers of samples of a similar type need to be analysed. The observation that n.i.r. calibrations are extremely sample specific is a consequence of a mathematical approach to calibration in which the instrument is considered a black box and n.i.r. and reference data are related to each other by multiple regression analysis. Using this method it is easy to find different calibration constants and even different wavelengths for each set of samples. On the other hand by selecting an absorption band as the measurement wavelength together with one or more reference wavelengths to correct for the overall level of the spectrum and for any bands overlapping the absorption band, universal calibrations for protein and moisture in wheat[2,3] and flour[4,5] were obtained. A multipurpose food laboratory, however, requires even more general

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calibrations so an experiment was undertaken to test the feasibility of a single n.i.r. calibration for moisture in samples which differ very widely in their physical characteristics.

2 EXPERIMENTAL

2.1 Samples

Forty white flours milled from grists varying between 100% English and 100% Canadian Western Red Spring (CWRS) were collected from nine commercial flour mills in the UK and Eire. Six UK home-grown wheat cultivars (Avalon, Hobbit, Sentry, Copain, Prince and Aquila) were each conditioned to seven moisture levels and a CWRS sample was conditioned to six moisture levels. One aliquot of each of the wheats was ground using a Udy Cyclone mill fitted with 0·5 mm screen. Thus, a total set of 136 samples comprising 40 white flours, 48 wheats and 48 whole wheats was used; this set was then divided randomly into equal numbers of calibration and prediction samples. Reference moisture contents were determined by ICC Method 110/1.

2.2 N.i.r. reflectance measurement

N.i.r. data at 1940 and 2310 nm were obtained as previously described[4] using a Technicon InfraAlyzer 300B instrument. N.i.r. spectra were recorded using a Pacific Scientific Mk I 6350 Research Composition Analyzer.[6]

2.3 Statistical analysis

Multiple regression analysis of reference moisture contents on n.i.r. data was carried out for each of the three separate calibration sets (white flour, ground wheat, whole wheat) and for the combined set. Each calibration was then tested against the corresponding prediction set using the procedure detailed in ICC Recommendation 202.

3 RESULTS AND DISCUSSION

3.1 Selection of wavelengths

The proposed method of obtaining more general n.i.r. calibrations involves selection of wavelengths on chemical grounds prior to multiple regression analysis. The measurement wavelength is chosen to correspond with an absorption band in the n.i.r. spectrum of the substance to be determined and water has a band at 1940 nm corresponding to the combination of O—H stretch with O—H bend;[7] this band is present in the n.i.r. spectrum of a cereal food such as flour and is removed by oven drying (Fig. 1). 1940 nm is therefore chosen as the measurement wavelength for n.i.r. moisture calibrations involving such samples. Since Fig. 1 shows that the band at 1940 nm does not overlap with any other bands, all that is required of the reference wavelength is to correct for the background. However, the method of background correction in reflectance spectroscopy

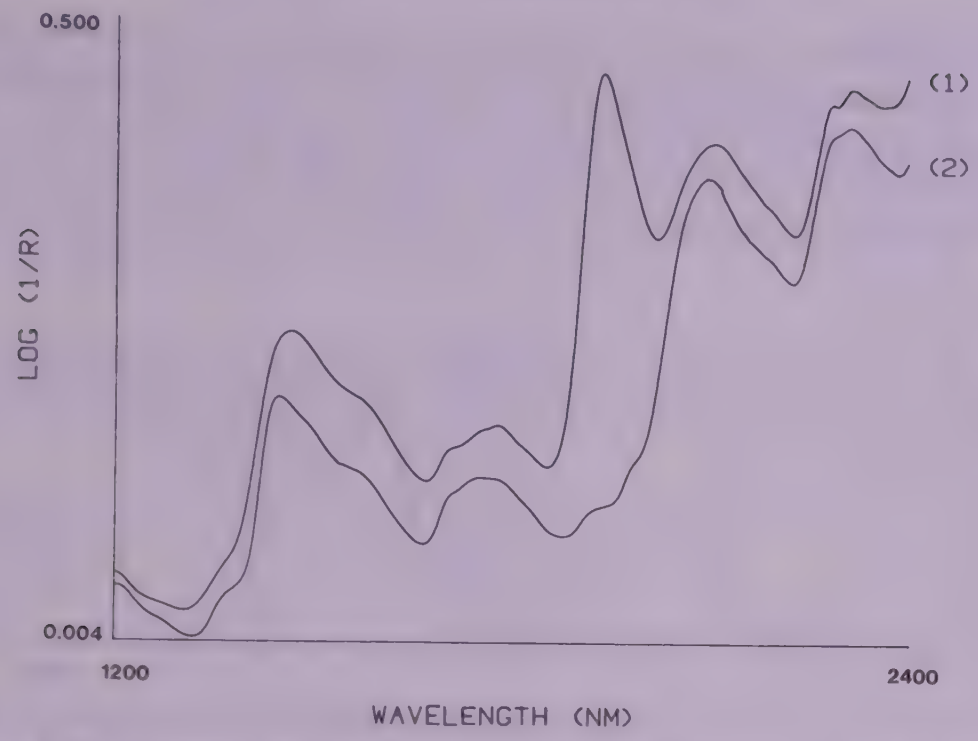

Fig. 1. N.i.r. spectra of a flour sample, before (1) and after (2) oven drying.

differs from that in more conventional transmission spectroscopy. N.i.r. reflectance spectroscopy is based on the relationship

$$\log (1/R) = \frac{ac}{s}$$

where R is the reflectance, a the absorptivity, c the concentration of the substance to be determined and s the scatter coefficient. Log $(1/R)$ for a given concentration of absorber therefore depends on s which in turn is influenced mainly by the particle size of a powder. Hence this property has a pronounced effect on the overall level of the spectrum, as shown in Fig. 2, and for foods the particle size is not constant but varies from sample to sample.

It has been shown that the particle size effect is proportional to the magnitude of log $(1/R)$[8] or in other words two wavelengths at which log $(1/R)$ is equal have equal values for s. Therefore to cancel the particle size effect, a reference wavelength having a mean log $(1/R)$ for the calibration samples as close as possible to that of 1940 nm is chosen. Of the filters available in the InfraAlyzer 300B, only 2310 nm fulfils this requirement and the mean log $(1/R)$ values at 1940 and 2310 nm for the combined calibration set were 0·71061 and 0·70940 respectively.

In previous reports on the n.i.r. determination of moisture in ground wheat[2] and flour[4,5] a three-term calibration equation involving log $(1/R)$ at 1940, 2310 and 2230 nm was proposed. This equation was selected by studying minimal adequate subsets (a statistical method for finding equations with the smallest number of terms which fit the calibration data as well as the full equation) and choosing the one which contained the 1940 nm measurement wavelength.

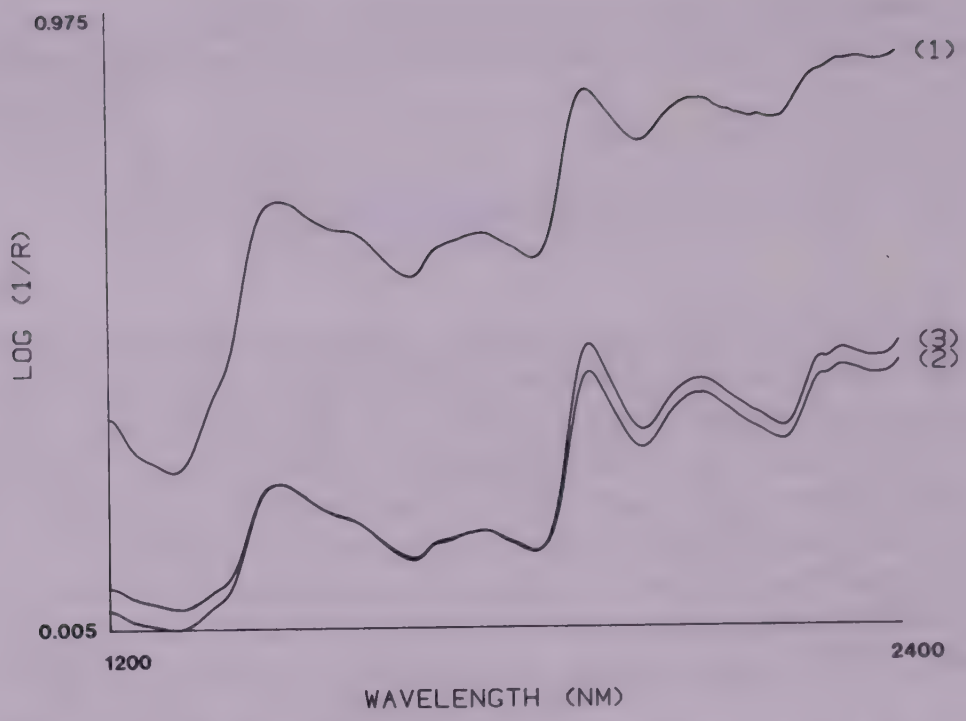

Fig. 2. N.i.r. spectra of whole wheat (1), ground wheat (2) and white flour (3).

However, it is not simple to make statistical comparisons between equations with different numbers of terms so it would not be surprising if the wavelengths selected mathematically differed from those selected on a theoretical basis.

3.2 Evaluation of calibrations

The mean spectra of whole wheat, ground wheat and white flour (Fig. 2) show that although these sample types differ widely in their physical characteristics, especially particle size, the n.i.r. spectra have essentially the same shape but are displaced along the log $(1/R)$ axis. According to the theory set out above, it should be possible therefore to effect a calibration for moisture covering all these samples by measurement of log $(1/R)$ at 1940 and 2310 nm. Table 1 shows the calibration data obtained by performing separate calibrations for the three sample types and a combined one of the general form

$$\% \text{ moisture} = K_0 + K_1(L_{2310}) + K_2(L_{1940})$$

where L_{2310} and L_{1940} refer to the log $(1/R)$ values at 2310 and 1940 nm respectively.

TABLE 1
N.i.r. Calibration Data for Moisture in White Flour, Ground Wheat and Whole Wheat

Sample set	*No. of samples*	*Range (%)*	s_c *(%)*	K_0	K_1 *(2310 nm)*	K_2 *(1940 nm)*
White flour	20	12·3–14·6	0·12	14·38	−89·83	86·75
Ground wheat	24	10·8–13·6	0·11	11·40	−68·97	71·56
Whole wheat	24	12·3–17·8	0·29	11·76	−74·50	77·23
Combined	68	10·8–17·8	0·24	10·82	−72·29	75·93

The figure for the residual standard deviation (s_c) for the combined calibration was satisfactory compared with those for the individual calibrations both in the present studies and in the literature.[1] The values for the constants K_1 and K_2 for ground and whole wheats were not significantly different ($P<0{\cdot}05$) despite the large differences in mean log $(1/R)$ and despite the two sets having different ranges of moisture content.

TABLE 2
N.i.r. Prediction Data for Moisture in White Flour, Ground Wheat and Whole Wheat

Sample set	*No. of samples*	*Range (%)*	s_p *(%)*	*Mean difference reference – n.i.r.*
White flour	20	11·5–14·9	0·12	−0·005
Ground wheat	24	10·5–13·8	0·15	+0·032
Whole wheat	24	12·3–18·0	0·19	+0·051
Combined	68	10·5–18·0	0·22	+0·004

Table 2 shows the validation results for the calibrations given in Table 1. There are no significant biases ($P<0{\cdot}05$) and although the standard deviation of differences between n.i.r. and oven moisture, s_p, for the combined calibration is significantly greater ($P<0{\cdot}05$) than those for the individual calibrations for flour and ground wheat it is nevertheless acceptable for routine use. Theoretically, when an absorption band is being corrected to the level of the spectrum the calibration constants should have a sum of zero. In this example the effect of particle size on log $(1/R)$ at 2310 nm and 1940 nm is not equal hence $-K_1$ and K_2 do not have exactly the same magnitude.

4 CONCLUSIONS

The results presented in this paper demonstrate that, for moisture at least, a single calibration with acceptable performance can be obtained using a theoretical approach to selection of wavelengths for samples which differ very widely in their physical characteristics. It must be admitted, however, that moisture is a simple case and application of the proposed method of wavelength selection would not always be straightforward.

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Yield, Selected Chemical Composition and Nutritive Value of 14 Selections of Amaranth Grain Representing Four Species

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ABSTRACT

A set of 14 selections of four amaranth species were studied. Six yielded over 10 kg 36 m^{-2}, and three below 5 kg 36 m^{-2}. Grain size varied from 1·55 to 2·14 mm, and seed weight from 0·46 to 1·18 mg $seed^{-1}$. There was no relationship between seed weight and yield. Protein content varied from 12·5 to 16·0%, while fat varied from 7·7 to 12·8%. The content of P, K, Ca, Mg, Na, Fe, Cu, Mn and Zn was similar among all selections. Trace amounts of C14 fatty acids were found, while C16 acids varied from 16·83 to 23·83% of the oil. The C18:0 fatty acids varied from 1·86 to 4·11%, the C18:1 from 20·29 to 35·46%, while the C18:2 fatty acids varied from 38·25 to 57·86%. Lysine varied from 0·73 to 0·84%, with tryptophan values ranging from 0·18 to 0·28%. Seeds from all selections were processed by hot-water soaking for 20 min followed by drum-drying, for protein quality evaluation.

The three A. caudatus *had an average protein efficiency ratio (PER) of 2·45;* A. hybridus *a PER of 2·34;* A. cruentus *2·36 and* A. hypochondriacus *2·33. Differences were not statistically different. Light and dark coloured seeds had the same average value of 2·36. the study showed important genetic differences in chemical composition.*

Key words: Amaranth, chemical composition, amino acid content, protein quality.

1 INTRODUCTION

Chemical information on amaranth grain is relatively available;[1–4] however, due to the agronomic potential of this ancient crop[1,5] and to its exceptionally high nutritive value,[6–8] additional information, particularly related to agronomic performance, is useful as a means to strengthen its potential as a food or a feed resource. Becker *et al.*,[2] reported on a compositional study of 10 amaranth grain samples representing single species or crosses between species grown under different agronomic conditions which according to the authors contributed significantly to the variability found. Saunders and Becker[1] recently reviewed the seed chemistry of amaranth and indicated that variations in grain composition grown under diverse conditions are considerable. This variability is evident from various studies which have been reported, particularly germ plasm coming from wide areas,[6] (Bressani *et al.*, unpublished); however, variability is also due, and may be even more important, to genetic make-up, as evidenced in a study of 25 selections of *A. caudatus* grown in one locality (Imeri, A. G. *et al.*, unpublished). With respect to nutritive value as established by biological assays, differences are obtained when the raw grain is fed;[5] however, these differences disappear to a large extent in processed samples[5] (Bressani, R. *et al.*, unpublished; Imeri, A. G. *et al.*, unpublished). The first observation, on raw grain, is probably due to unknown factors or nutrient bioavailability limiting animal growth, while the second is probably due to essential amino acid pattern (Bressani, R. *et al.*, unpublished). In this report, the selected partial composition of 14 amaranth grain selections representing four species is reported. All 14 selections were planted in one locality, thus, the differences in chemical composition should be due exclusively to genetic make-up. The nutritive quality of the protein of processed samples is also reported.

2 EXPERIMENTAL

The amaranth grain selections used in this study were sent to INCAP by Rodale, Kutztown, PA. They were received in late 1982 and planted at the INCAP Experimental Farm facilities in June 1983. The experimental farm is located at an altitude of 1500 m above sea level, with temperature fluctuations varying from 12 to 24°C. Rainfall occurs from May to October and total amounts average 1140 mm. The soils are heavy clays which become water saturated in August–September and begin to dry in late October. Each selection was planted in experimental plots 3×3 m with four replicates each, in a completely randomised block design. The number of plants per 9 m^2 was the same for all replicates and for all selections. During their development, plant height at flowering and at harvest was established. The identification of each selection, its species, grain colour, origin and plant type are presented in Table 1.

At harvest, the influorescences were cut off by hand and thrown into the hopper of a thresher for grain separation and collection was done on a per-replicate basis. The seed size and seed weight per replicate for each selection were measured on 100 seeds. Moisture, protein, ash and ether extract content were determined by AOAC procedures[9] on samples from each replication. Pooled samples from all

TABLE 1
Identification of Samples

INCAP No.	*Rodale No.*	*Seed colour*[a]	*Amaranth species*	*Origin*	*Plant type*
1	A-73	C	*Caudatus*	Cusco, Perú	South American grain
2	A-982	O	*Caudatus*	Apurimac, Perú	South American grain
3	A-1113	C	*Caudatus*	Ayacucho, Perú	South American grain
4	82S-1004	O	*Hybridus*	Pakistan	Prima grain
5	82-1011	O	*Cruentus*	Mexico	Mexican grain
6	82S-434	C	*Cruentus*	Mexico	Mexican grain
7	82S-1034	O	*Cruentus*	Dahomey	African grain
8	A-718	O	*Hypochondriacus*	Oaxaca, Mexico	Aztec grain
9	A-720	O	*Hypochondriacus*	Oaxaca, Mexico	Aztec grain
10	82S-1023	C	*Hypochondriacus*	Mexico	Mercado grain
11	81S-1024	O	*Hypochondriacus*	Mexico	Mercado grain
12	82S-674	O	*Hypochondriacus*	Unknown	Spitee
13	82S-SP130	C	*Hypochondriacus*	Nepal	Nepal grain
14	82S-1008	C	*Hypochondriacus*	India	Nepal grain

[a]Seed colour: C=light; O=dark.

four replications were used for mineral analysis by atomic absorption spectrometry on the ash of samples weighing 1·0 g. Likewise, fatty acid content was determined by gas chromatography on pooled samples from all four replicates per selection. Lysine content was determined by paper chromatography of 6 N HCl hydrolysates[10] and tryptophan by the method of Villegas *et al.*[11].

For the biological assay of protein quality, 2·5 g of grain were soaked in water for 20 min and then passed through a double-drum drier at 2 rev min^{-1} using steam at 4·2 kg cm^{-2} pressure to heat the drums. The resulting product was ground and analysed for protein by the Kjeldahl method.[12] Diets were prepared by using processed amaranth flour to provide 10% protein. It was supplemented with 1% cod liver oil, 5% refined cottonseed oil, and 4% mineral mixture.[12] All diets were adjusted to 100% with refined maize starch and all were supplemented with a complete vitamin mixture at 5 ml 100 g^{-1} added in liquid form.[13]

Each diet was fed *ad libitum* to weanling rats (Wistar strain from INCAP's animal colony, eight rats per group), housed individually in all-wire screen cages with raised screen bottoms. Water was available all the time and feed intake and weight changes were measured every 7 days.

The data were statistically analysed using standard methods as described by Snedecor and Cochran.[14]

3 RESULTS AND DISCUSSION

3.1 Yield and grain size

Information on the development of the plant and the total yield for the four species is presented in Table 2. Days to flower varied from 26 to 92, at plant heights from 22 to 145 cm. *A. hypochondriacus* showed greatest variability in this respect, *A. caudatus* being least variable. Days to harvest, and plant height at harvest varied from 80 to 146 days, and from 75 to 280 cm, respectively. As before, *A. hypochondriacus* showed the largest variability in plant height at harvest. The total yield per selection, expressed as g 36 m^{-2} also indicate large

TABLE 2
Some Agronomic Characteristics of the Amaranth Grain Selections Studied

Selection	Days to flower	Height to flower (cm)	Days to harvest	Height at harvest (cm)	Yield (g 36 m^{-2})
1. *A. caudatus* (A-713)	61	110	129	240	3,093
2. *A. caudatus* (A-982)	61	100	129	210	12,755
3. *A. caudatus* (A-1113)	61	96	120	230	11,883
6. *A. hybridus* (82S-1004)	26	23	80	75	4,921
7. *A. cruentus* (82S-1011)	33	30	90	160	11,296
8. *A. cruentus* (82S-434)	34	56	90	190	13,206
9. *A. cruentus* (82S-1034)	63	28	95	150	10,960
4. *A. hypochondriacus* (A-718)	92	145	146	280	14,728
5. *A. hypochondriacus* (A-720)	61	140	129	260	9,759
10. *A. hypochondriacus* (82S-1023)	33	25	80	130	9,759
11. *A. hypochondriacus* (82S-1024)	33	25	80	140	8,791
12. *A. hypochondriacus* (82S-674)	26	24	80	90	7,905
13. *A. hypochondriacus* (82S-SP130)	33	27	80	90	9,624
14. *A. hypochondriacus* (82S-1008)	26	22	80	75	4,937

differences between selections. Yield varied from 3·093 to 14·728 g 36 m^{-2}. In this respect, *A. cruentus* showed less variability than the other two species. Statistical analysis of the yield data indicated differences to be highly significant. Plant height at flowering was highly correlated with plant height at harvest (r=0·9013), but plant height at flowering and yield were not related (r=0·3671), while some relationship was found between plant height at harvest and yield (r=0·4659<0·05). The difference between plant height at harvest and plant height at flowering was also not correlated with yield (r=−0·3897). The best yielding cultivar was *A. hypochondriacus* (A-718) with 4091 kg ha^{-1}, suggesting the high yield potential of amaranth.

Table 3 summarises size and weight of the seed of the 14 selections. The differences in both characteristics between cultivars of all species were statistically significant. Seed size varied from 1·55 to 2·14 mm and seed weight varied from 0·46 to 1·18 mg $seed^{-1}$, values which were similar to those reported for *A. caudatus* (Imeri, A. G. *et al.*, unpublished) and other species.[1,5] Seed weight was not correlated with yield as reported before (Imera, A. G. *et al.*, unpublished). Selection of cultivars of all species for higher seed size and weight is recommended, since it will make harvest easier with a cleaner seed. However, higher seed size should not affect yield or protein content.

TABLE 3
Grain Size and Weight

Selection	*Size (mm)*	*Weight (mg seed^{-1})*
1. *A. caudatus* (A-713)	1·55	0·46
2. *A. caudatus* (A-982)	1·71	0·66
3. *A. caudatus* (A-1113)	1·75	0·72
6. *A. hybridus* (82S-1004)	1·93	0·85
7. *A. cruentus* (82S-1011)	1·88	0·82
8. *A. cruentus* (82S-434)	1·92	0·84
9. *A. cruentus* (82S-1034)	2·11	0·52
4. *A. hypochondriacus* (A-718)	1·69	0·68
5. *A. hypochondriacus* (A-720)	1·58	0·53
10. *A. hypochondriacus* (82S-1023)	2·12	1·14
11. *A. hypochondriacus* (82S-1024)	2·14	1·18
12. *A. hypochondriacus* (82S-674)	1·98	0·93
13. *A. hypochondriacus* (82S-SP130)	1·89	0·93
14. *A. hypochondriacus* (82S-1008)	1·95	1·02

3.2 Chemical composition

Table 4 summarises the partial proximate composition of the samples studied. Differences in ether extract, protein and ash between cultivars were statistically significant. With respect to ether extract, *A. caudatus* had, on average, a higher content than *A. cruentus* and both had a higher content than *A. hypochondriacus*. This group, however, had one selection with the lowest lipid content in this study. With respect to protein content the *A. cruentus* selections were slightly higher in this nutrient than the other three species. Fat content was positively correlated with yield ($r=+0{\cdot}2912^{*}$) while protein content was negatively related to yield ($r=-0{\cdot}4127^{*}$). This last relationship is common to cereal grains as well as food legumes, and breeding programmes should select or breed materials, which, if

TABLE 4
Moisture, Ether Extract, Protein and Ash Content of 14 Amaranth Grain Selections (%)

Selection	*Moisture*	*Ether extract*	*Protein*	*Ash*
1. *A. caudatus* (A-713)	11·72	12·50	14·77	2·94
2. *A. caudatus* (A-982)	12·12	12·35	12·55	2·65
3. *A. caudatus* (A-1113)	11·62	11·55	12·50	3·02
6. *A. hybridus* (82S-1004)	13·25	8·47	15·60	5·01
7. *A. cruentus* (82S-1011)	11·92	12·85	14·70	2·83
8. *A. cruentus* (82S-434)	12·27	10·90	15·07	3·18
9. *A. cruentus* (82S-1034)	13·32	9·20	16·00	3·36
4. *A. hypochondriacus* (A-718)	12·10	10·45	13·70	3·33
5. *A. hypochondriacus* (A-720)	11·90	10·35	14·75	3·93
10. *A. hypochondriacus* (82S-1023)	12·77	10·60	15·32	2·94
11. *A. hypochondriacus* (82S-1024)	12·85	9·17	14·75	3·91
12. *A. hypochondriacus* (82S-674)	13·55	8·25	15·07	3·42
13. *A. hypochondriacus* (82S-SP130)	12·77	9·17	15·05	3·28
14. *A. hypochondriacus* (82S-1008)	12·42	7·72	15·60	3·99

*Statistically significant at 0·05

yielding more, should not affect protein content. With respect to seed size, this was not associated with protein content ($r = +0{\cdot}2244$); however, seed size and fat content were negatively related ($r = -0{\cdot}4193^*$). Finally, protein and ether extract were negatively related ($r = -0{\cdot}4645^*$). Since both protein and fat content make amaranth attractive from the nutritional point of view, breeding and selection programmes for yield should consider minimum levels of these two nutrients. These could not be less than 15% for protein and not less than 8% for fat.

The fatty acid composition of the 14 cultivars studied are presented in Table 5. The values presented are similar to those published by other authors and reviewers.[1,2,15,16] The variability within species was as high as between species, being more evident for C18:0 and C18:2 than for C16:0 fatty acids. Since all materials were grown in the same locality and under the same agricultural practices, these differences are more of a genetic than of an environmental or agronomic nature. Amaranth's high linoleic acid content is obviously an attractive nutritional characteristic, and together with its high protein and oil content, make it a significant resource in human feeding as has been indicated before.[8,16] Oil digestibility, however, has been reported to be around 92%, which is slightly lower than other edible oils (Garcia, L. A., unpublished). Table 6 summarises the

TABLE 5
Fatty Acid Content of 14 Amaranth Grain Selections (%)

Selection	*C14:0*	*C16:0*	*C18:0*	*C18:1*	*C18:2*	*(C18:3+ C20:0)*
1. *A. caudatus* (A-713)	Tr.	17·18	1·86	25·17	51·57	3·17
2. *A. caudatus* (A-982)	Tr.	20·09	2·60	28·71	46·75	0·95
3. *A. caudatus* (A-1113)	Tr.	18·63	2·53	28·54	47·39	2·01
6. *A. hybridus* (82S-1004)	Tr.	19·01	2·92	22·23	53·29	1·40
7. *A. cruentus* (82S-1011)	Tr.	16·83	2·75	35·46	43·13	1·00
8. *A. cruentus* (82S-434)	0·88	21·58	4·00	34·46	38·25	—
9. *A. cruentus* (82S-1034)	Tr.	21·17	4·11	25·81	48·92	—
4. *A. hypochondriacus* (A-718)	Tr.	17·71	2·25	20·29	57·86	0·43
5. *A. hypochondriacus* (A-720)	Tr.	19·66	2·70	22·35	53·28	1·23
10. *A. hypochondriacus* (82S-1023)	Tr.	21·33	3·11	26·37	48·25	—
11. *A. hypochondriacus* (82S-1024)	Tr.	22·10	2·96	25·10	49·83	—
12. *A. hypochondriacus* (82S-674)	Tr.	21·56	2·91	22·96	52·56	—
13. *A. hypochondriacus* (82S-SP130)	Tr.	22·94	3·07	24·44	49·55	—
14. *A. hypochondriacus* (82S-1008)	Tr.	23·83	3·34	22·94	48·84	—

*Statistically significant at 0·05.

TABLE 6
Mineral Content of 14 Amaranth Grain Selections (mg 100 g^{-1})

Selection	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
1. *A. caudatus* (A-713)	518	493	246	396	19	20	0·85	3·39	3·40
2. *A. caudatus* (A-982)	594	532	201	270	22	15	0·87	2·62	3·49
3. *A. caudatus* (A-1113)	597	571	205	290	24	28	0·85	2·56	3·41
6. *A. hybridus* (82S-1004)	565	532	303	344	26	104	4·10	5·18	3·45
7. *A. cruentus* (82S-1011)	589	545	202	334	24	17	1·68	2·51	4·19
8. *A. cruentus* (82S-434)	544	518	263	311	24	34	1·69	3·38	4·22
9. *A. cruentus* (82S-1034)	536	511	260	386	27	27	1·71	4·27	4·27
4. *A. hypochondriacus* (A-718)	625	549	287	372	25	30	1·63	3·28	4·10
5. *A. hypochondriacus* (A-720)	667	556	256	368	26	100	3·51	4·39	4·39
10. *A. hypochondriacus* (82S-1023)	586	570	204	323	24	22	1·70	2·55	4·12
11. *A. hypochondriacus* (82S-1024)	556	590	222	333	24	55	2·56	4·27	3·42
12. *A. hypochondriacus* (82S-674)	589	538	308	359	22	26	1·71	3·42	3·42
13. *A. hypochondriacus* (82S-SP130)	576	516	206	309	22	31	1·72	2·58	3·43
14. *A. hypochondriacus* (82S-1008)	605	621	223	327	22	111	3·98	3·98	3·98

TABLE 7
Lysine and Tryptophan Content of 14 Amaranth Grain Selections

Selection	Lysine		Tryptophan	
	g%	g 16 g^{-1} N	g%	g 16 g^{-1} N
1. *A. caudatus* (A-713)	0·82	5·55	0·19	1·36
2. *A. caudatus* (A-982)	0·74	5·89	0·18	1·44
3. *A. caudatus* (A-1113)	0·80	6·40	0·20	1·70
6. *A. hybridus* (82S-1004)	0·84	5·38	0·20	1·45
7. *A. cruentus* (82S-1011)	0·82	5·58	0·28	1·85
8. *A. cruentus* (82S-434)	0·81	5·37	0·25	1·87
9. *A. cruentus* (82S-1034)	0·79	4·94	0·18	1·10
4. *A. hypochondriacus* (A-718)	0·77	5·62	0·23	1·79
5. *A. hypochondriacus* (A-720)	0·74	5·02	0·18	1·34
10. *A. hypochondriacus* (82S-1023)	0·81	5·29	0·21	1·51
11. *A. hypochondriacus* (82S-1024)	0·79	5·35	0·20	1·41
12. *A. hypochondriacus* (82S-674)	0·79	5·24	0·18	1·19
13. *A. hypochondriacus* (82S-SP130)	0·78	5·18	0·23	1·58
14. *A. hypochondriacus* (82S-1008)	0·73	4·68	0·20	1·37

mineral content of the amaranth cultivars studied. The values fall within values reported by other workers.[1,2] There seems to be little variation between cultivars of the same species and between species.

The lysine and tryptophan content of the samples of amaranth grain under study are shown in Table 7, expressed as weight percentage and on the basis of 100 g of protein. These amino acids were chosen because they are deficient in maize protein,[17] which could benefit from supplementation with amaranth grain. The results show that cultivars with higher protein contain slightly more lysine and tryptophan on a weight basis as has been shown before.[3] However, this relationship is more evident with lysine. The relationship becomes negative when the two amino acids are expressed on a protein basis. Although lysine and tryptophan levels are higher in amaranth than in cereal grain particularly, the amounts are not exceedingly high. Therefore, breeding or selection programmes when breeding for high yield should take lysine content which should not fall below a value of 400 mg g^{-1} N on the basis of an 85% true protein digestibility. This is an important consideration since lysine content in amaranth is one of the positive nutritional attributes of this grain.[1,8]

3.3 Protein quality

Table 8 summarises the biological evaluation of the 14 cultivars of amaranth assayed on processed samples. Statistical analysis of weight gain, food intake and PER showed that no two groups are significantly different at the 0·050 level of significance. Even though protein quality values are not statistically different, PER varies from 2·23 to 2·59 for all samples. Among *A. caudatus*, the range is between 2·38 and 2·59, for *A. cruentus*, between 2·23 and 2·55, and for *A. hypochondriacus*, between 2·23 and 2·41, smaller than for the other species. These values are 81–94% of the value of casein, making amaranth protein quality exceptionally high as has been reported before[5] (Bressani, R. *et al.*, unpublished; Bressani, R. *et al.*, unpublished; Imeri, A. G. *et al.*, unpublished). Protein content of the samples was found to be negatively related to protein quality ($r = -0{\cdot}5035$). However, this is not uncommon since it is also the case for cereal and legume foods that are deficient in essential amino acids.[18] Usually as protein content is higher, the content of the limiting amino acid on a per gram of protein basis becomes smaller.[19–21]

In the present case, no significant relationship was found between lysine percentage, on a weight basis, and PER; however, the relationship became stronger when lysine was expressed as g 16 g^{-1} N with a correlation coefficient of $r = +0{\cdot}4397$ (NS). With respect to tryptophan, the relationship to PER, both as weight percentage or on the basis of protein was positive but not statistically significant.

The importance of yield per unit area of land is, of course, the most important objective for amaranth as it is for all cereal grains and food legumes. However, in achieving such an objective it is also important to consider the nutrient content levels so that the high nutritional quality of amaranth is kept, particularly if intended for human feeding.

TABLE 8
Protein Quality of 14 Selections of Amaranth Grain

Selection	*Seed colour*[a]	*Weight gain (g)*[b]	*Food intake (g)*[b]	*PER*[b]
1. *A. caudatus* (A-713)	C	107±12·7	403±34·7	2·39±0·23
2. *A. caudatus* (A-982)	O	113±24·9	390±55·9	2·59±0·29
3. *A. caudatus* (A-1113)	C	118±17·8	415±41·7	2·38±0·17
6. *A. hybridus* (82S-1004)	O	114±13·5	440±44·0	2·34±0·17
7. *A. cruentus* (82S-1011)	O	108±19·4	417±56·0	2·55±0·17
8. *A. cruentus* (82S-434)	C	117±25·1	438±60·4	2·31±0·22
9. *A. cruentus* (82S-1034)	O	106±27·9	460±70·6	2·23±0·26
4. *A. hypochondriacus* (A-718)	O	108±10·7	424±34·8	2·30±0·16
5. *A. hypochondriacus* (A-720)	O	100±15·6	411±42·6	2·37±0·16
10. *A. hypochondriacus* (82S-1023)	C	113±9·9	428±25·5	2·38±0·17
11. *A. hypochondriacus* (82S-1024)	O	105±17·9	445±46·7	2·23±0·23
12. *A. hypochondriacus* (82S-674)	O	113±16·1	452±41·8	2·29±0·14
13. *A. hypochondriacus* (82S-SP130)	C	117±19·3	445±67·5	2·41±0·08
14. *A. hypochondriacus* (82S-1008)	C	119±29·4	453±63·3	2·32±0·27
Casein				2·74

[a]Colour: C=light; O=dark.
[b]Average±SD.

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Wheat-Bug Damage in New Zealand Wheats. Development of a Simple SDS-Sedimentation Test for Bug Damage

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ABSTRACT

Lines of three varieties of New Zealand wheat (cv. Aotea, Oroua and Kopara) damaged by wheat-bug were investigated. Hydrolysis of gluten proteins was shown to be of the endo- rather than the exo-proteolytic type. Electrophoresis revealed that the bug protease had a high specificity for the high molecular weight (HMW) glutenin subunits. An autolytic assay method was developed based on the decrease in sodium dodecyl sulphate (SDS)-sedimentation volume for bug-damaged flours when they were incubated in distilled water for 30 min at 37°C. This method was specific for bug damage and exhibited no interference from other grain defects such as heat damage, field sprouting and laboratory germination.

Key words: Wheat, wheat-bug, proteolytic enzymes, gel electrophoresis, high molecular weight (HMW) glutenin subunits.

1 INTRODUCTION

Pre-harvest insect attack on grain, or bug damage, is widespread in Europe, Asia and North Africa.[1–5] Bug damage is usually associated with insects of the genera *Eurygaster* and *Aelia*. During the course of their feeding these insects inject a digestive secretion into the grain and withdraw digested nutrients. Some of the digestive juices remain in the grain and persist in the resultant flour after milling.

The consequence of this enzymic material in the baking process is to bring about rapid relaxation of the dough and breakdown of the gluten structure. This results in a dough which is runny and sticky, and produces a characteristic loaf of poor volume and texture. This phenomenon was first reported by Berliner in

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1931,[1] and its biochemistry has since been studied in some depth.[6–14] The deleterious effects on dough proteins have been shown to be due to the presence of proteolytic enzymes.

Similar damage to grain by insects was first reported in New Zealand in 1936,[15] and investigations carried out at that time suggested that the insects responsible may be *Nysius huttoni* (White) and *Hudsona anceps* (White) (natives), and *Stenotus binotatus* (Fabr.). Meredith investigated the biochemistry of bug damage in New Zealand and concluded that technological problems resulting from bug damage were due to enzymic reduction of gluten proteins, rather than proteolysis.[16]

The present study was designed to determine the nature of the gluten degrading enzyme and to develop a small scale test for its presence in grain and flour.

2 MATERIALS AND METHODS

2.1 Wheats

Three bug-damaged samples detected by the Wheat Research Institute harvest evaluation testbake were examined. These were: one line of the red-grained wheat Oroua (OBD) grown in Mid-Canterbury in the 1983/84 season, one of the red-grained wheat Kopara (KopBD) grown in Central Otago in the 1985/86 season, and one line of the white-grained wheat Aotea (ABD).

Laboratory-sprouted wheat was prepared by soaking wheat in distilled water for 2 h, followed by germination under a damp cloth at room temperature for varying periods of time. After germination the wheat was air-dried to 14–15% moisture.

Heat-damaged wheat was produced by heating wheat (16% moisture) in sealed containers for 16 h at temperatures from 50 to 100°C. The wheat was then air-dried to 14% moisture.

All other wheats used were selected from the Wheat Research Institute harvest evaluation file.

2.2 Proteolytic activity

Exoprotease activity was determined by the modified Ayre-Anderson method as described by Hanford,[17] except that 6% rather than 5 M trichloroacetic acid was used as the precipitant. Soluble nitrogen was determined by the ninhydrin method of Yemm and Cocking,[18] as adapted by Hanford.[17]

Gluten-softening enzyme activity was determined according to the method of Hanford,[17] with the firmness of a gluten ball incubated at 30°C being assessed at intervals and scored on a subjective scale of 8 (very firm) to 0 (rotten, almost liquid).

2.3 Sodium dodecyl sulphate (SDS) soluble protein

One gram of flour was suspended in 10 ml of distilled water, 10 ml of 3% (w/v) SDS in distilled water was added and the mixture shaken periodically for 10 min. Extracts were clarified by centrifuging at 12 000×*g* for 15 min and filtered through

Whatman No. 5 filter paper. Protein content of 0·1 ml aliquots was determined by the Coomassie dye binding method of Bradford.[19]

2.4 SDS-sedimentation test

SDS-insoluble protein was determined on 5 g of flour or 6 g of wholemeal using the SDS-sedimentation test according to Axford *et al.*[20]

A modified SDS test was also employed. It involved incubation of the flour or wholemeal in 50 ml of distilled water in a 100 ml measuring cylinder for 30 min at 37°C. The cylinder was shaken for 15 s every 5 min. After the final shake 50 ml of SDS–lactic acid was added and the remainder of the procedure was carried out as described by Axford *et al.*[20]

2.5 Acidic polyacrylamide gel electrophoresis (A-PAGE)

Gliadin proteins were extracted from flour or crushed grain with 1 M urea (6 ml g^{-1}) and 10 μl aliquots separated on 2·5–27% polyacrylamide gels (Gradient Laboratories Pty Ltd, Pyrmont, NSW, Australia) in sodium lactate buffer (pH 3·1) according to the methods of Du Cros and Wrigley.[21]

To determine the effects of autolysis, flours or wholemeals were incubated in distilled water (3 ml g^{-1}) for 1 h at 37°C. Autolysis was quenched by addition of 2 M urea (3 ml g^{-1}). Extraction was carried out for 30 min before 10 μl of clarified extract was applied to the gel.

2.6 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Total wheat proteins were extracted from flour or crushed grain and separated on a 17% polyacrylamide gel according to the method of Payne *et al.*[22]

To determine effects of autolysis, flours or wholemeals were incubated in distilled water (4 ml g^{-1}). Autolysis was quenched by addition of an extracting medium containing 8% w/v SDS, 5% v/v 2-mercaptoethanol, 20% v/v glycerol, 0·002% w/v bromophenol blue and 0·124 M tris–HCl, pH 6·8 (4 ml g^{-1}).

3 RESULTS AND DISCUSSION

3.1 Proteolytic activity of bug-damaged flours

Table 1 gives the results of exo- and endo-protease activities as defined by Hanford.[17] From these results it is obvious that the active component is not of the exo-proteolytic type, as less free amino acids are liberated from the bug-damaged flour than from a sound flour. It is worth noting that two of the damaged flours (ABD and OBD) had very high blank values, indicating high levels of free amino acids in the grain. These high levels of amino acids may be due to the bug attack halting or slowing the physiological development of the grain. Visual inspection of damaged kernels indicated that they were generally very vitreous and somewhat shrivelled, this may indicate that bug damage has caused interruption of the later stages of grain filling.

The gluten-softening test demonstrated a clear distinction between damaged and undamaged wheats. The gluten from the damaged wheats liquified rapidly.

TABLE 1
Proteolytic Activity of Flours

A. Exoprotease[a] (increase in soluble nitrogen; ninhydrin method)

Flour	*Zero time*	*3 h*	*Difference*
Sound	0·277 (0·264, 0·290)	0·525 (0·514, 0·535)	0·248
ABD	0·604 (0·607, 0·601)	0·750 (0·741, 0·759)	0·146
OBD	1·003 (1·048, 0·958)	1·095 (1·087, 1·103)	0·092
KopBD	0·342 (0·335, 0·348)	0·473 (0·454, 0·491)	0·131

B. Gluten softening[b] (8, very firm; 0, rotten, almost liquid)

Flour	*Zero time*	*1 h*	*2 h*
Sound	4	2	2
ABD	2	0	—
OBD	4	0	—
KopBD	3	0	—

[a]Figures quoted are absorbance values at 570 nm. Figures in parentheses are experimental values; differences are given as the difference between average values.
[b]From single determinations.

Hanford noted that Russian wheats damaged by the wheat-bug (*Eurygaster* or *Aelia*) exhibited high gluten-softening activity, but only medium production of soluble nitrogen. Using his nomenclature the active protease secreted by the Russian wheat-bug would be classified as an α-proteinase. Hanford suggests that these α-proteinases may be either endo-peptidases or reductases. The results in Table 1 show a similar pattern of activity for bug damage in New Zealand wheats.

3.2 Electrophoretic studies

In an attempt to discover what effect bug damage had on gluten proteins electrophoretic separation of gliadin and total flour proteins was carried out, before and after incubation of the flour in water at 37°C.

3.2.1 Effect of bug damage on gliadin protein composition

The electrophoregrams of the gliadin proteins from damaged and undamaged samples of the three varieties demonstrated no obvious differences (Fig. 1), suggesting the damage caused by the insect saliva has little effect on the structure of this class of proteins. This could be seen as an indication that the damage is through a reductase, as the gliadin proteins do not form inter-molecular disulphide bonds and the intra-molecular disulphide bonds may be inaccessible to attack by reducing enzymes.

Electrophoregrams of incubated, bug-damaged samples do show an extra component with mobility lower than the slowest moving gliadins (ω-gliadins). This is presumably a degradation product resulting from hydrolysis of proteins of higher molecular weight (glutenins).

3.2.2 Effect of bug damage on total protein composition

The SDS-PAGE electrophoregram of total proteins shows a clear effect of bug

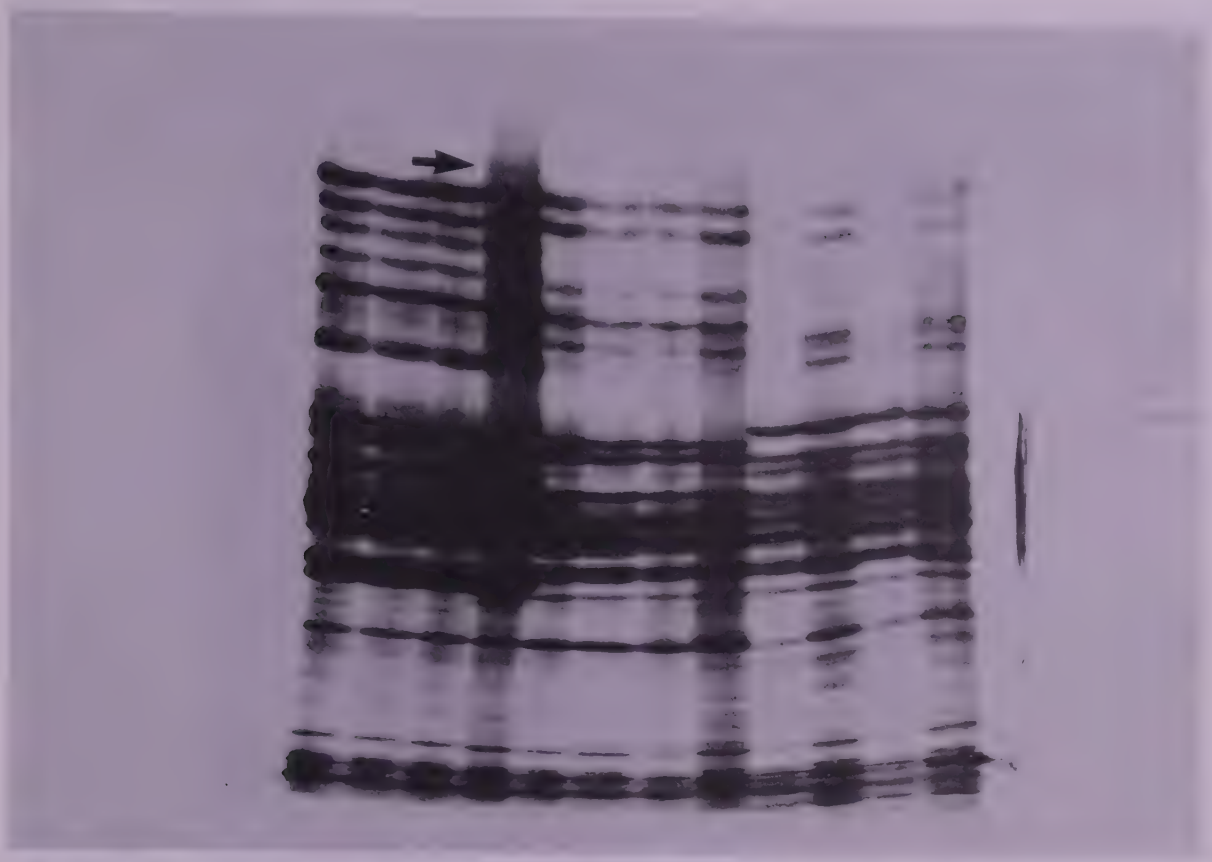

Fig. 1. Effect of bug damage on gliadin proteins, separated by A-PAGE. Unincubated and incubated (1 h, 37°C) alternate from left to right. Samples, in pairs from the left, are: Aotea standard, Aotea bug damaged, Oroua standard, Oroua bug damaged, Kopara standard and Kopara bug damaged. The arrow indicates the position of a glutenin degradation fragment.

damage (Fig. 2). The lowest mobility components, the high molecular weight subunits of glutenin, decrease in staining intensity with flour incubation. Native glutenin has been hypothesised to exist as a polymer of subunits, with the high molecular weight components forming the backbone of the polymer.[23,24] The observed degradation of these subunits is inconsistent with the hypothesis of a reductase, or reducing substance as the protein degrading component.[16] Such an action would presumably have a similar effect on the proteins to the mercaptoethanol used in the extraction regime for SDS-PAGE, and would reduce the disulphide linkages which bond the subunits to one another, but would leave the subunits themselves intact.

It appears more likely that the active component is an endo-protease with a high specificity for a pair, or sequence of amino acids which occur in glutenin, but not in gliadin. Shewry *et al.*[25] recently reviewed current knowledge of wheat storage protein amino acid sequences, as determined by direct sequencing or nucleotide sequencing. They point out that the repeat sequences in the high molecular weight (HMW) glutenins are very different from the repeat motifs in all other storage proteins.

The observed selective degradation of glutenin revealed by SDS-PAGE appears similar to that reported by Yakovenko *et al.*,[13] for Russian wheats damaged by the chinch bug. In the case of the chinch bug the selectivity of the enzyme is explained in terms of the greater resistance of the gliadin proteins to

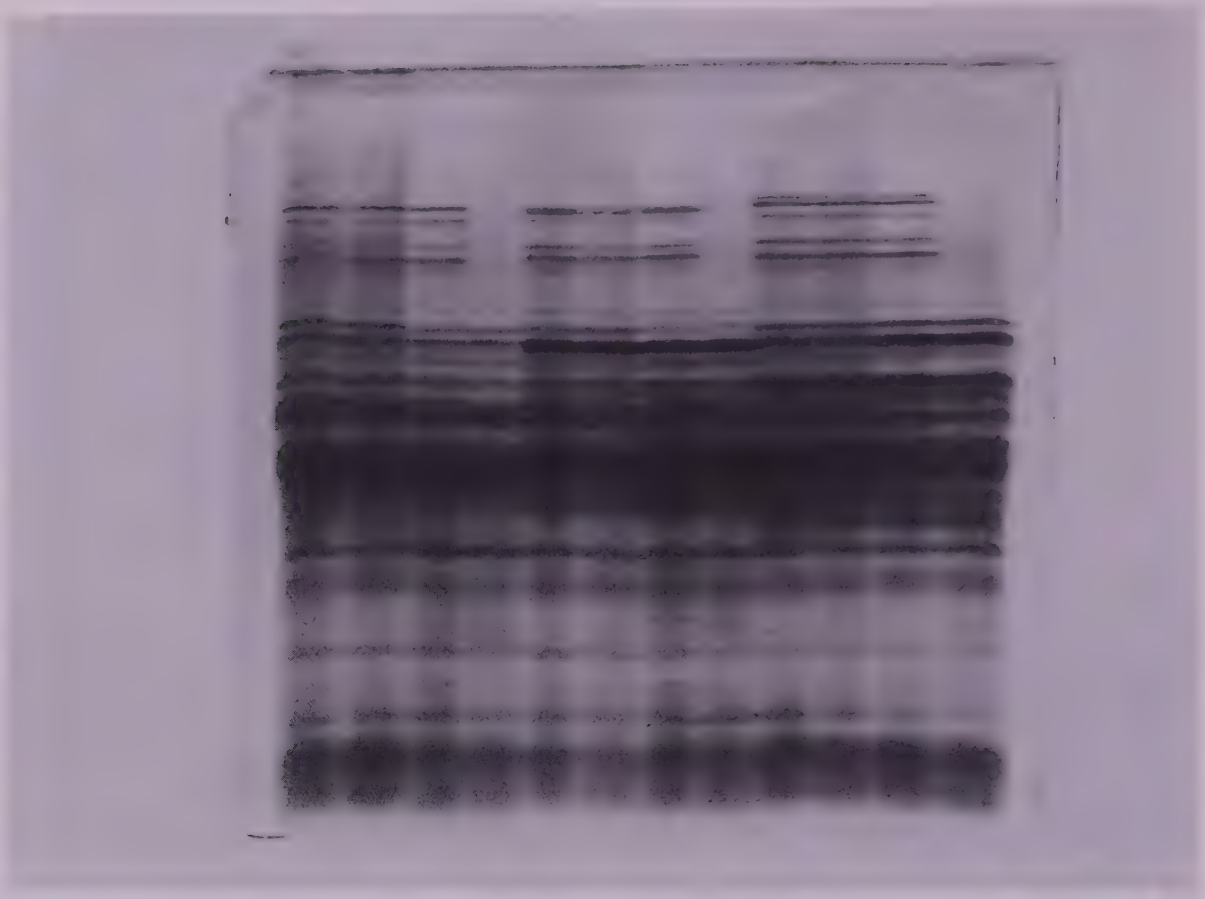

Fig. 2. Effect of bug damage on total proteins, separated by SDS-PAGE. Unincubated and incubated (1 h, 37°C) alternate from left to right. Samples, in pairs from left, are: Aotea standard, Aotea bug damaged, Oroua standard, Oroua bug damaged, Kopara standard and Kopara bug damaged. The HMW glutenin subunits are the distinct group of four to five bands at the top of the gel.

proteolysis. Glutenin degradation products were observed by Yakovenko *et al.*,[13] with mobilities similar to α-gliadin. The only degradation product so far observed in our study has a much lower electrophoretic mobility (similar to ω-gliadin), suggesting some difference in mode of enzyme action.

3.3 Tests for bug damage

3.3.1 Increase in SDS-soluble protein

Polymeric glutenin is characterised by its insolubility in solutions of SDS. Therefore, it was decided to monitor the course of the enzyme reaction by observing the increase in SDS-soluble polypeptides. Preliminary studies suggested that an incubation time of 30 min at 37°C was optimal for an assessment of proteolytic activity by this method. Table 2A shows the results of SDS-soluble polypeptides as measured by the Coomassie dye-binding method. There is a significantly greater increase in the amount of SDS-soluble material after incubation for the bug-damaged samples than for the sound samples. This confirms the results suggested by SDS-PAGE.

3.3.2 Decrease in SDS-sedimentation volume

An alternative approach is to measure the amount of SDS-insoluble protein remaining after incubation. SDS-insoluble glutenin can be measured simply by

TABLE 2
Proteolytic Activity of Flours

A. The increase in SDS-soluble protein after incubation at 37°C for 30 minutes[a]

Flour	*Zero time*	*30 min*	*Difference*
Sound	0·205 (0·201–0·212)	0·217 (0·210–0·220)	0·012
ABD	0·282 (0·279–0·286)	0·334 (0·331–0·340)	0·052
OBD	0·440 (0·439–0·441)	0·551 (0·524–0·567)	0·111
KopBD	0·281 (0·279–0·282)	0·344 (0·339–0·354)	0·063

B. The decrease in SDS-sedimentation volume after incubation at 37°C for 30 min[b]

Sample	*Standard method (ml)*		*30 min incubation (ml)*		*Difference (ml)*	
	Flour	*Wholemeal*	*Flour*	*Wholemeal*	*Flour*	*Wholemeal*
Sound	57		57		0	
ABD	35		9		26	
OBD	66	63	50	39	16	24
KopBD	36	38	8	13	28	25

[a]Figures quoted are absorption values for the protein–dye complex at 595 nm, and are the average of triplicate determinations. Figures in parentheses show the range of experimental values.
[b]Volumes are averages of duplicate determinations. Duplicates were all within 2 ml of one another.

the SDS-sedimentation test. This test determines the height of sediment in a measuring cylinder after a set weight of flour is dispersed in an SDS–lactic acid solution and allowed to sediment. The conventional test involves 4 min dispersion of the flour in water at 22°C before addition of the SDS–lactic acid solution. Initial experiments suggested that the length and temperature of dispersion in water were not critical for sound flours.

Table 2B gives the SDS-sedimentation volumes for flours and wholemeals, as assessed by the conventional test and for the modified test, involving incubation of the flour in water for 30 min at 37°C. This is similar to the Zeleny sedimentation test method used by Greenaway *et al.*[26] to detect stinkbug damage in wheat, except they employed a much longer hydration time (3 h) to detect proteolytic activity.

Both flour and wholemeal tests give a good indication of bug damage although the relationship between flour and wholemeal results is not consistent.

3.3.3 Specificity of the SDS-sedimentation test for bug damage

In order to determine whether other cereal defects would interfere with this test wholemeals from a range of sound (good and poor quality), field-sprouted, laboratory-sprouted and heat-damaged samples were analysed. The results are summarised in Table 3. There was a spread of volume changes; however, several general comments can be made. For sound flours there was an average volume drop of 1·4 ml. This could be due to the presence of natural wheat proteases;

TABLE 3
Difference in SDS-Sedimentation Volume Between Incubated and Unincubated Wheaten Wholemeals[a]

Type of wheat	*No. of samples*	*Range of volume differences (ml)*	*Average difference (ml)*
Sound	23	−13–10	−1·4
Field sprouted	16	−15–11	−4·9
Laboratory sprouted	3	9–12	5·3
Heat damaged	4	0–11	5·8
Bug damaged[b]	3	−26–−24	−25·0

[a]All SDS-sedimentation volumes were the average of duplicates, which were within 4 ml of one another.
[b]Wholemeal SDS-sedimentation values were not available for ABD; flour values have been substituted for this Table.

however, this decrease is not significant. The average drop for field sprouted grain was greater (4·9 ml), which suggests that germination has been accompanied by an increase in glutenin-degrading proteolytic activity. By contrast, laboratory-germinated samples demonstrated an average increase in SDS volume. No obvious explanation for this phenomenon is apparent at this stage. In the heat-damaged samples all proteolytic activity would presumably have been destroyed and the increase in sedimentation volume upon incubation may have been due to more effective dispersion of the wheatmeal in the water. The most severely heat-damaged sample (16 h at 100°C) had a much lower SDS-sedimentation volume than the other heat-damaged samples, but this did not decrease further after incubation.

The bug-damaged samples analysed here showed dramatic decreases in sedimentation volume upon incubation. On the basis of these results a drop in sedimentation volume of over 20 ml would be a strong indication of bug damage. If any doubt existed a repeat of the experiment using a longer incubation time should clarify the issue.

3.4 Comparison of bug damage in New Zealand wheats to bug damage in other countries

It appears that *Nysius huttoni* is probably the insect responsible for bug damage in New Zealand wheats (Cressey, P. J., unpublished). *Nysius* is native to New Zealand; however, some of the results obtained so far suggest some similarities between the damage it causes and the damage caused by insects in other countries.

Greenaway *et al.*[26] used a method similar to the SDS-sedimentation test reported here, but based on the Zeleny sedimentation test, to detect stinkbug (*Eurygaster* or *Aelia*) in Austrian wheats. However, sedimentation tests would presumably be applicable to determining glutenin degradation by a wide range of proteolytic enzymes.

Hanford[17] observed that the Russian wheat bug produced high glutenin

softening activity, with only moderate production of soluble nitrogen. This is in agreement with our observations for bug damage in New Zealand wheats.

Koz'mina and Tvorogova[6] reported that autolysis of chinch bug damaged wheat resulted in an increase in 0·05 M acetic acid soluble protein, indicating decomposition of the acetic acid-insoluble glutenin. This was confirmed by the electrophoretic study of Yakovenko *et al.*,[13] who concluded that chinch bug damage proceeded through breakdown of glutenin to components with similar electrophoretic mobility to α-gliadin. While the selective degradation of glutenin appears similar to bug damage in New Zealand wheats the degradation products reported appear in different electrophoretic mobility classes. This suggests that the enzymes responsible may have differing degrees of specificity, or different points at which they hydrolyse the protein chains.

Kretovich[9] determined the pH optimum for protease from bug-damaged wheat as being neutral or slightly alkaline (pH 7–8). The enzyme present in New Zealand wheat has a higher optimum, at about pH 9·0 (Cressey, P. J., unpublished).

While there are some similarities between bug proteases reported in Russia and Europe, and those investigated in the present study, there appear to be sufficient differences to suggest that the New Zealand problem involves a novel proteolytic enzyme. This enzyme requires further characterisation to determine the mechanism by which it hydrolyses wheat glutenin.

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Determination of Silver in Foods by Neutron Activation Analysis

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ABSTRACT

Silver has been determined in a number of foodstuffs by neutron activation analysis. The results range from <1 to 530 ng g^{-1} dry matter and are highly variable, suggesting that random contamination of samples may occur. Higher concentrations of up to 31 μg g^{-1} dry matter were found in wild fungi. From these results, supplemented by others, the British dietary intake is estimated to be 4·5 μg Ag day^{-1}, which constitutes no hazard to human health.

Key words: Diet, food, fungi, neutron activation analysis, silver.

1 INTRODUCTION

Silver is an element which has rarely been determined in foodstuffs. Techniques with adequate sensitivity to determine existing levels include plasma emission spectrometry,[1] solvent extraction coupled with atomic absorption spectrometry[2,3] and neutron activation analysis.[4] The only foods which have been found to accumulate silver to any extent are certain fungi.[5–7]

In the present work the technique of radiochemical neutron activation analysis (NAA) has been applied to determine silver in miscellaneous foods and a few fungi. The accuracy of the method has been tested by analysing a number of certified reference materials.[8]

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2 EXPERIMENTAL

2.1 Samples

Samples of foodstuffs were mostly supplied by the Plant Sciences department at Reading University (Nos. 001–010) and by the Laboratory of the Government Chemist (Nos. 106–908). Wild fungi were collected locally.

2.2 Apparatus

Gallenkamp Oven set at 85°C.
Gallenkamp Muffle Furnace set at 500°C, with a silica liner.
Phillips 250W Infra-red lamp.
MSE Centrifuge.
Nuclear Data Pulse height analyser with Ge(Li) gamma ray detector head.
Neutron activation was carried out in the University of London research reactor at Ascot, Berks.

2.3 Reagents

All chemicals used were of AR grade, or 'Specpure' for silver nitrate:

Ammonia, 19 M.
Ferric nitrate, 5% w/v.
Hydrochloric acid, 2 M.
Nitric acid, 8 M and 16 M.
Silver nitrate carrier, 20 mg Ag g^{-1} in 2 M nitric acid.
Silver nitrate standard, 10 mg Ag g^{-1}, freshly prepared.
Sodium sulphide, 2 M.

2.4 Methods

Samples were laid on polythene sheets which had been cleaned by soaking in 8 M nitric acid for 5 h and well rinsed with water. They were dried to constant weight at 85°C. Five grams dry matter was weighed in a silica crucible and heated at 500°C for 10 h. The ash was then sealed into a polythene packet. Spiking with ^{110m}Ag showed that losses of silver during this procedure were negligible.

Standards were made by weighing one or two drops of the standard silver nitrate solution on a small square of polythene film. The drops were evaporated to dryness under an infra-red lamp, and the film was folded and sealed into a packet. Packets containing standards and samples were activated in a flux of about 10^{12} neutrons cm^{-2} s^{-1} for about 60 h, and then left to decay for about 14 days.

The radiochemical separation of silver from samples and standards has been described in detail.[8] After adding 20 mg silver carrier, the ash was dissolved in 10 ml 16 M nitric acid; occasionally a few drops of 18 M sulphuric acid or 30% hydrogen peroxide were needed to aid the dissolution. The nitric acid was evaporated off and the silver was precipitated as silver chloride. This was dissolved in ammonia and scavenged with a precipitate of iron(III) hydroxide. The silver was then precipitated as silver sulphide, dissolved in nitric acid, reprecipitated as sulphide, dried, weighed and counted. Counting usually required 22 h

and the areas under the gamma peaks at 0·66 and 0·88 MeV were obtained from a microcomputer. The counts were corrected for any weight losses of the silver carrier during the separation.

3 RESULTS

Results of the analyses are given in Table 1 in concentrations of silver g^{-1} dry matter (DM). The parts analysed were the edible portions used for human consumption.

TABLE 1
Silver Content of Dry Foods

Code No.	*Nature (origin)*	*ng Ag g^{-1} DM*
001	Rice, *Oryza sativa* (Pakistan)	43·6
002	Barley, *Hordeum vulgare* (Britain)	<1
003	Sorghum, *Sorghum bicolor* (Argentina)	95·4
004	Sorghum, *Sorghum bicolor* (Sudan)	158
005	Sesame, *Sesamum indicum* (Sudan)	197
006	Potato, *Solanum tuberosum* (Britain)	9·6
007	Carrot, *Daucus carota* (Britain)	48·7
008	Maize, *Zea mays* (Britain)	18·6
009	Broad Bean, *Vicia faba* (Britain)	3
010	Bean, *Phaseolus* sp. (Britain)	6·2
011	False Death Cap, *Amanita citrina* (Ascot)	144
012	Tawny Grisette, *Amanita fulva* (Ascot)	17700
013	Fly Agric, *Amanita muscaria* (Ascot)	5470
014	Boletus, *Boletus bovinus* (Ascot)	321
015	Bay Boletus, *Boletus badius* (Ascot)	4110
016	Brown Birch Boletus, *Boletus scaber* (Crowthorne)	874
017	Mushroom, *Agaricus bitorquis* (Shinfield)	31400
635	Carrot, frozen (Welwyn)	8
636	Carrot, frozen (Welwyn Co-op)	4
637	Carrot, frozen (Eltham)	64·6
638	Carrot, frozen (Wimbledon)	13·6
639	Carrot, frozen (Wimbledon)	74·5
640	Carrot, frozen (Greenwich)	35
642	Spinach, frozen (Eltham)	53·9
643	Spinach, frozen (Welling)	66
644	Spinach, frozen (Eltham)	9·3
645	Spinach, frozen (High Wycombe)	232
710	Spinach, frozen (Sainsbury)	199
711	Spinach, frozen (Sainsbury)	27·7
713	Bread, white (Independent)	<1
717	Bread, white (Sainsbury)	<1
719	Bread, white (Clarkes)	<1
720	Bread, white (Broomfields)	<1
722	Bread, wholemeal (Independent)	<1
725	Bread, white (Hovis)	<1
726	Bread, white (Vitbe)	<1
728	Bread, white (Tesco)	278
729	Biscuits (Sainsbury)	<1

(*continued*)

TABLE 1—*contd.*

Code No.	*Nature (origin)*	*ng Ag g^{-1} DM*
730	Biscuits (Tesco)	<1
732	Biscuits (Waitrose)	<1
733	Biscuits (Crawford)	<1
885	Beef (Tesco)	<1
887	Beef (Plumstead)	25·9
892	Lamb (Eltham)	4·5
895	Lamb (Tesco)	<1
898	Pork (Eltham)	527
900	Pork (Wimbledon)	2·2
905	Chicken quarters (London)	93·8
908	Chicken quarters (London)	14·9
106	Chicken liver (Healeys)	23·7
107	Chicken liver (Healeys)	56·9
108	Chicken liver (Sainsbury)	5·5
121	Chicken liver (Banham)	13·4

4 DISCUSSION

4.1 Comments

One conclusion that can be drawn from Table 1 is that apart from the fungi, none of which is commonly used as food in Britain, the silver contents of the foods are very low. Another conclusion is that the variation from sample to sample may be large and unpredictable. Compare for example the two pork samples, or sample 728 with all other bread samples analysed.

Silver contents of plant tissues must ultimately depend on silver supplied by the soil, while silver contents of animal tissues should be related to the intake of silver in the diet. The present results suggest, however, that at these very low concentrations, random contamination of foods is significant. Such contamination can readily arise from the use of silver cutlery or jewellery, or from dusts arising from photographic materials, and we believe that contamination has occurred before samples 728, 898 and probably 905 were analysed. Indeed, without 'clean room' facilities for preparing samples, all the samples studied may have been contaminated, though they do represent what people actually eat.

These results on the accumulation of silver by fungi, especially *Agaricus* spp., confirm the observations of others.[5–7] Since soils contain 100–400 ng Ag g^{-1},[9] the value of 31 μg g^{-1} Ag found in *Agaricus bitorquis* represents a concentration factor of about a hundredfold. The mechanism of, and the reasons for, this accumulation of silver are unknown, though it may be that some fungi produce an argentophilic ligand to neutralise the toxic effects of the silver(I) cation in the soil.

4.2 Estimation of silver in British diets

In Table 2 an estimate is made of the daily intake of silver in the British diet. Values for the fresh weight (FW) of each type of food consumed per day are taken from the UK total diet survey,[10] modified to give a greater intake of beverages.[3] Mean silver contents for cereals, meats and vegetables were calculated from the

TABLE 2
Estimating Daily Input of Silver in Foods and Diet

Food group	*gFW eaten day^{-1}*	*ng Ag gFW^{-1}*	*Dietary intake μg Ag^{-1} day^{-1}*
Cereals	230	0·7	0·161
Meat	150	2·2	0·330
Root vegetables	180	2·4	0·432
Other vegetables	110	11·3	1·243
Fruit	170	8·0[3]	1·360
Fish	20	4·0[3]	0·080
Fats	80	2·0[3]	0·160
Milk	400	0·5[3]	0·200
Other beverages	600	0·8[3]	0·480
Total	1940		4·446

data in Table 1 after rejecting outliers. Silver contents of other foods were taken from reference 3. It seems that vegetables and fruit contribute about 50% of the British dietary intake of silver.

This estimate of a daily intake of silver of 4·5 μg agrees with estimates of 8·7, 9 and 7 μg for British,[3] Swedish[11] and Italian diets.[12] It is somewhat lower than values of 40, 70, 27 and 27 μg reported respectively for diets in the USA,[13,14] Britain[15] and Canada.[4] The chances of contamination and analytical error are strong when studying silver. The element is not known to be essential for any form of life, and the toxic effects of any of the above intakes are unlikely to be measurable Hence it is not anticipated that any adverse consequences to human health will be caused by the silver present in current diets.

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Total, Non-volatile Free Fatty Acids as a Freshness Index for Hake (*Merluccius hubbsi*) Stored in Ice

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ABSTRACT

Total, non-volatile free fatty acids (FFA) content was determined in hake muscle during fish storage in ice. FFA increased linearly in every season according to freshness loss as determined by sensory scoring. FFA determination is proposed as a valuable alternative to sensory scoring in determining fish deterioration in ice.

Key words: Hake, *Merluccius hubbsi*, freshness index, ice storage, free fatty acids.

1 INTRODUCTION

Hake freshness has been traditionally evaluated through sensory assessments.[1] Recent developments to replace sensory scoring by physical or chemical methods have been introduced.[2] These methods, however, do not provide enough sensitivity in the first 5–10 days of storage and the detected changes are too small to follow the storage life of hake stored in ice. A good improvement seemed to be the viscosity determination of soluble muscle protein extracts taken from hake stored in ice.[3] This parameter, however, is subject to non-linear seasonal variations.[4]

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The increase in total non-volatile free fatty acids (FFA) has been correlated with anchovy ripening in brine,[5] a process during which extensive changes in chemical compounds other than lipids occur.[6] The main objective of this work was to study the possibility of using total, non-volatile FFA release in fish muscle as a non-sensory freshness index for hake stored in ice.

2 EXPERIMENTAL

Hake (*Merluccius hubbsi*) was caught from fishing grounds on the Argentinian platform in the South Atlantic Ocean during all seasons, and over a period of 2 years from 1979 to 1981. Fish samples were obtained from commercial vessels and kept in ice after catching and throughout the storage period studied in each experiment.

From time to time at least six specimens were taken at random from different boxes, and sensorily evaluated by a panel of trained people.[2]

Fillets obtained from each specimen were taken off and minced to obtain a uniform muscle paste, to be allotted to dry weight and total, non-volatile FFA determinations.

Twenty grams of six minced fillets were homogenized with 50 ml ethanol in a Braun MRA homogenizer. Homogenates were filtered through Whatman No. 1 filter paper, and 0·1 ml aliquots of the filtrate used to measure FFA according to the method described by Smith.[7] Calibration curves were obtained for each analysis set, by using palmitic acid (C16:0) as the standard. FFA determinations were done in quadruplicate. Results were expressed as milliequivalents of palmitic acid per 100 g wet tissue. Data points in Figs 1–3 are mean values of at least six samples.

3 RESULTS AND DISCUSSION

Hake freshness was evaluated on fish kept in ice through the sensory scoring method developed by Lupin *et al.*[2] Figure 1 shows sensory assessment versus days in ice during different seasons, and up to 18 days total storage time.

A similar linear response was obtained when muscle total non-volatile FFA content versus storage time in ice was determined, whatever the season (Fig. 2).

When FFA levels in hake muscle and sensory scores from fish kept in ice were depicted together, a good correlation could be obtained (Fig. 3).

Lack of trained people to form the sensory panel on some occasions and a failure of FFA determinations, due to unforeseen events, account for the unmatched points depicted in Figs 1 and 2.

The evidence presented here shows that FFA level in muscle could be used instead of the subjective sensory scoring method, in determining hake freshness. This has a triple significance. First, since a linear correlation between FFA and storage time has been obtained (Fig. 2), FFA level could be used to calculate how many days hake has been kept in ice after catching. Second, since a sensory score 3–3·5 is the borderline for freshness acceptability,[2] and this corresponds to 0·4–0·5

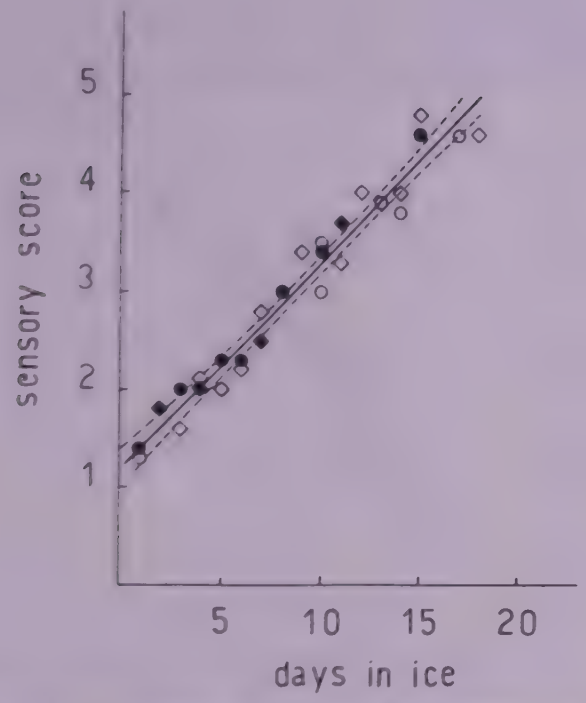

Fig. 1. Time course of sensory scores in hake stored in ice during different seasons. □, summer; ■, fall; ○, winter. ●, spring. Each point represents samples taken from at least six different specimens. Solid line represents regression line for sensory scores versus days in ice. Dashed lines represent confidence limits for $P<0{\cdot}05$.

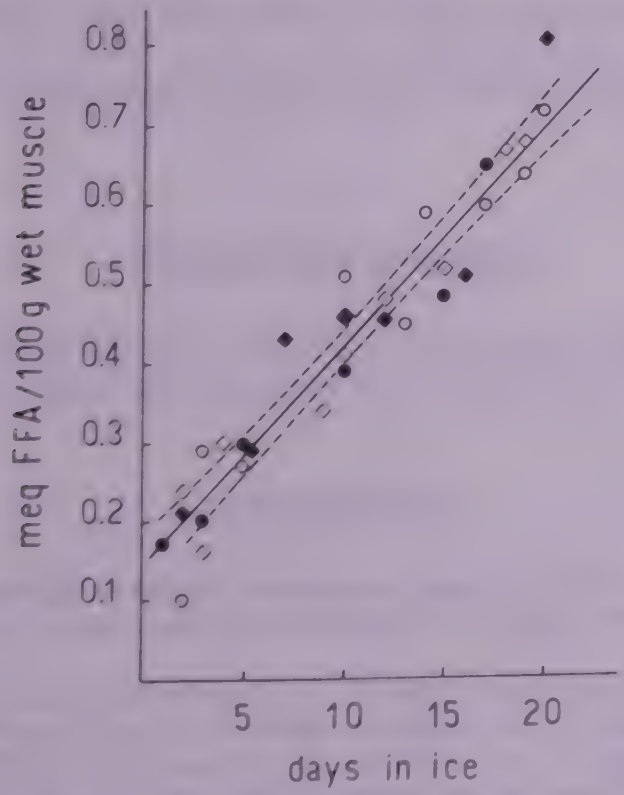

Fig. 2. Time course of total, non-volatile FFA in hake stored in ice during different seasons. Solid line represents regression line for FFA versus days in ice. See Fig. 1 for symbols.

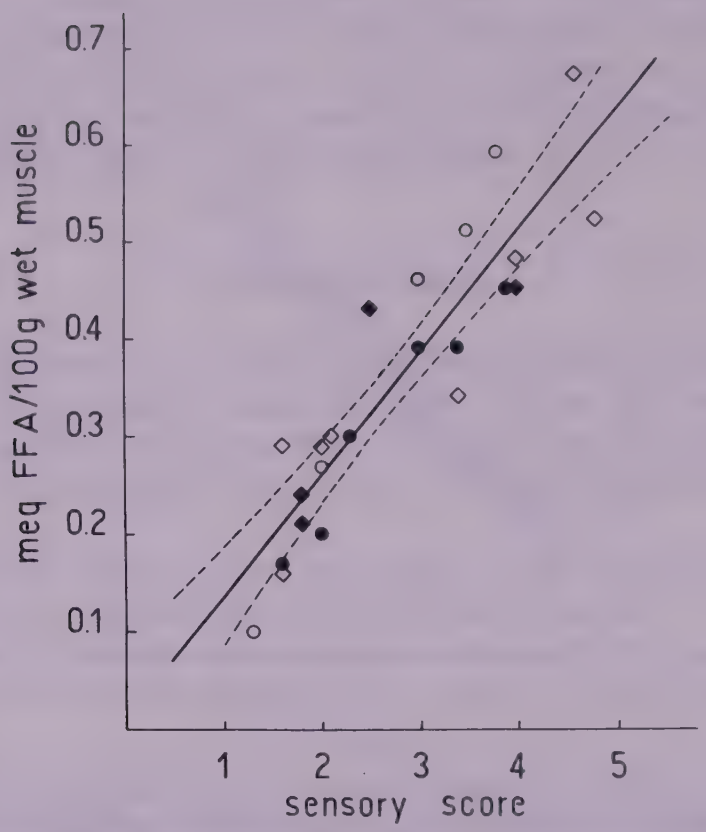

Fig. 3. Total, non-volatile FFA versus sensory scores for hake stored in ice during different seasons. Solid line represents regression line for FFA versus sensory scores. See Fig. 1 for symbols.

FFA meq %, the latter values could be used as the borderline for hake consumption and processing, independently of the season. Third, FFA could serve to control freshness in fillets, where sensory scoring is less appropriate than in whole fish.

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Three α-Gliadins of Cappelle-Desprez

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ABSTRACT

The strong α-doublet running behind the fastest major α-gliadin of Cappelle-Desprez on gel electrophoresis has been separated into three proteins. They were named: slow, middle and fast (S, M and F) from their mobilities on alkaline gels. The amino acid compositions were typical of gliadins, with no free thiol groups. Molecular weights and % amidation were: S31 000, 96; M32 000, 94; F29 000, 91. No convincing evidence for a glycoprotein nature was found. At acid pH the order seems to be, in decreasing mobility, F, S, M.

Key words: α-gliadin, Cappelle-Desprez, wheat, protein separation.

1 INTRODUCTION

Since work began on deducing amino acid sequences of gliadins[1] particularly from the base sequences of their genes,[2] the separation and partial characterisation of any further components had been abandoned here. Lawrence and Payne,[3] however, reported the separation of an oligomer of glutenin, and some years ago it was noticed here on starch-gel electrophoresis (SGEP) that both components of the strong α-doublet behind the fastest main α-gliadin[4] of Cappelle-Desprez gave multiple bands when run on polyacrylamide gel electrophoresis (PAGE) at pH 8·9 in the presence of sodium dodecyl sulphate (SDS). Owing to the small amounts available after long fractionation no further work was done, but the suspicion that multi-chain proteins were present remained. Later a second try at separating these failed. This paper describes the results of a third attempt.

2 EXPERIMENTAL

Separation of proteins

The three components were isolated from Cappelle-Desprez gliadin, prepared by extracting flour with 700 ml litre^{-1} ethanol as described earlier.[5] During the course of the work changes were made to improve separation, but only the recommended method is given. All buffers throughout were 2 M in dimethylformamide.

Stage 1

CM-Trisacryl M column (220×25 mm). Load: 2 g of gliadin in starting buffer (100 ml). Gradient (2 litres total volume): 0·005–0·2 M sodium acetate, pH 5·2. The fraction shown by electrophoresis to be richest in α-doublet was combined from all eight runs.

Stage 2

Column: as in stage 1. Load: 1·4 g in starting buffer (75 ml). Gradient (2 litres): 0·025–0·05 M sodium acetate, pH 5·2. Fractions rich in α-doublet were collected.

Stage 3

Gel filtration on Ultrogel AcA 54. Column: 1100×22 mm. Buffer: 0·01 M acetic acid, 0·05 M NaCl. Load: 115 mg in buffer (10 ml). The α-doublet peak was combined from three runs.

Stage 4

CM-Trisacryl M column (140×25 mm). Load: 250–300 mg in starting buffer (18 ml). Gradient (1 litre): 0·03–0·045 M sodium acetate, pH 5·2. The diffuse peak was split into six fractions.

Stage 5

QAE cellulose (Sigma) column (70×25 mm). Load (30–50 mg) in starting buffer (5 ml). Gradient (1 litre): 0–0·034 M NaCl in 0·01 M glycine–NaOH buffer, pH 10·4.

Some fractions were further purified by repeating Stage 5.

Amino acid analysis

Proteins were hydrolysed in 6 M HCl at 105°C *in vacuo* (~0·05 mmHg) for 1, 2 and 3 days. After evaporation of the HCl at room temperature the residues were taken up in loading buffer, pH 2·2, and spin filtered (0·2 μm) before being loaded on an automatic amino acid analyser. Duplicate runs were made on each hydrolysate, and corrections made for labile or slowly released amino acids.

SH determination

Protein (~0·5 mg) was treated with iodoacetamide (2–4 moles per mole of protein) in 0·33 M Tris HCl, pH 8·6, 9 mM EDTA, 8 M urea (0·4 ml). After 15 min in the dark the mixture was acidified with acetic acid, dialysed in the dark

for 24 h with four changes against ~0·01 M acetic acid, freeze-dried, hydrolysed for 1 day and analysed as above.

Tryptophan determination

Protein (~0·5 mg) was treated with a solution of 3 M *p*-toluenesulphonic acid, 2 g litre^{-1} tryptamine (0·2 ml) in a small tube (50×5 mm i.d.). The tube was drawn out, frozen, evacuated, then sealed as melting began. After hydrolysing at 110°C for 1 day the top was cut off and the contents transferred to another tube (9 mm i.d.). Thymol blue indicator (~20 μl) was added and the contents titrated to an orange colour with 2 M NaOH. The liquor (50 μl) was loaded on the analyser, after spin filtration.

Sugars

These were estimated semi-quantitatively by paper chromatography on Whatman No. 20 paper, after hydrolysis with 1 M H_2SO_4 as described.[6]

Molecular weights

These were measured from a calibration curve of the relative mobilities of standard proteins on SDS-PAGE under conditions of Laemmli and Favre.[7]

Alkaline gel electrophoresis

The method of Davis[8] for PAGE was used in a slab apparatus instead of tubes, and the gels were 6 M in urea.

Starch gel electrophoresis

The method was as used before but without urea.[9]

Free carboxyl groups

A carbodiimide caused free COOH to form a peptide bond with glycine.[10] Extra glycine was measured after hydrolysis.

3 RESULTS AND DISCUSSION

α-doublet separation

Kasarda *et al.*[2] report that separation of a-gliadin into five or so components is extremely difficult. Experience with these proteins confirms their view. The third attempt to resolve the α-doublet succeeded probably because of the high resolution of CM-Trisacryl M, which gave good yields of fairly pure doublet after only two stages. Gel filtration at the third stage eliminated substantial amounts of albumins, which can easily be missed because they run off the acid gels owing to the high mobility of the α-doublet. Low molecular weight glutenin was also removed at this stage, and was responsible for some of the bands that appeared on SDS-PAGE.

After a fourth stage of chromatography the doublet could be resolved into fractions containing varying proportions of the bands seen on acid gels (Fig. 1).

Fig. 1. Starch-gel electrophoresis (SGEP) of the three components of the α-doublet of Cappelle-Desprez gliadin (IA) at pH 3·7.

Fig. 2. Polyacrylamide gel electrophoresis (PAGE) in 6 M urea at pH 8·9 of the three components of the α-doublet of Cappelle-Desprez gliadin (IA). Unlabelled channels are fractions.

All these fractions contained three proteins that were well resolved on alkaline gels into bands labelled slow (S), middle (M) and fast (F) (Fig. 2). The doublet therefore had three components, which were separated on QAE-cellulose at pH 10·4, though to resolve M and S it was necessary to clip the leading and trailing edges of the first peak.

The products were pure enough for characterisation: the overloading of S shows up small amounts of a doublet in the M region; the faster is M and the slower is a gliadin that moves behind α-doublet on SGEP. On acid gels M appears to be the slow band whereas S and F have intermediate and fast positions respectively (Fig. 1). It was not easy to decide these positions owing to the nearness of the mobilities.

Compositions

The amino acid analyses (Table 1) show that S, M and F are gliadins with their high Glx, Pro, Leu and Ser, and low Lys. These are the first gliadins reported in which there are more Gly than Ala: usually Ala exceeds or equals Gly. No

TABLE 1
Amino Acid Compositions of Three α-Gliadins from Cappelle-Desprez (Residues Molecule^{-1})

	F	*M*	*S*
Asx	7	8	6
Thr	4	4	5
Ser	13	16	15
Glx	95	117	111
Pro	31	39	33
Gly	8	9	15
Ala	7	7	7
Cys	4	6	4
Val	11	12	12
Met	1	1	2
Ile	10	12	9
Leu	19	23	18
Tyr	7	4	7
Phe	14	8	11
His	7	8	7
Lys	2	2	2
Arg	3	4	4
Trp	2	1	1
Total	245	281	269
Mol. wt calculated from analysis	28 671	32 415	31 004
Av. mol. wt of anhydro amino acid	117·0	115·3	115·2
$\frac{\text{Ionic+polar}}{\text{nonpolar}}$	1·29	1·38	1·40
% amidation	91	94	96
$\frac{\text{Protein}}{\text{N}}$	5·6	5·4	5·4
Calculated molar absorption coefficient (ε) (litres mol^{-1} cm^{-1})	$2{\cdot}2\times10^4$	$1{\cdot}2\times10^4$	$1{\cdot}6\times10^4$

evidence for significant levels of SH groups was found. The amino acid compositions are generally similar to those of A-gliadins isolated by Kasarda,[11] though S has a high Gly and F and S appear to have only two disulphide bonds. It seemed that the proteins, like A-gliadins,[12] were not glycoproteins because none of the sugars known to be present in glycoproteins was found though, for reasons not understood, glucose at about one residue per protein molecule appeared in analyses of some preparations. It was found here that small amounts of glucose can come from QAE-cellulose eluant and from filter paper, but these sources could not account for the level of contamination. Residual glycolipid, as was found in crude gliadin by McMaster and Bushuk,[13] would not explain it either, because in that work galactose was the dominant sugar and glucose the second.

An unfractionated mixture of the three proteins did not have glucose, however, but there was an unexplained spot in the ribose area, which was absent from the purer fractions, which had faint spots beyond rhamnose.

Relative mobilities

Calculation of the relative charges on side chains at pH 8·9, that of the alkaline gel, gave +2 for S; −2 for M; −4 for F which agree with the order of mobilities at that pH.

At acid pH the net charge per unit of mass cannot be calculated accurately because it is not known whether the free side-chain COOH groups are on Asp or Glu or both. If all were on Glu and 3·7 was the pH in starch gel the net positive charges $\times 10^4$ per mass unit were 3·9 S, 3·8 M and 3·5 F. Though these figures are close, as are the mobilities in acid gels, that for F is out of order. Better agreement cannot be expected owing to the errors of amino acid analysis: an error of one basic residue per molecule (which is probable) in F could account for its anomalous figure. Analytical error is more likely to cause this than lack of purity: Ewart[6] derived expressions for the error in amino acid analysis caused by the presence of another protein. (These expressions unfortunately have the wrong sign, and should therefore be multiplied by −1.)

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